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The Stereoselective Reactivities of DL-Amino Acid *N*-Carboxyanhydrides for (−)-Menthol

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The stereoselective reactivities of some neutral DL-amino acid *N*-carboxyanhydrides for (−)-menthol were studied by varying the concentration of hydrogen chloride as a catalyst and the reaction temperature. From the results of gas-liquid-chromatographic analyses, the esterification rates of D-amino acids were shown to be higher than those of L-amino acids in all cases. The highest ratio of the esterification rate, V_D/V_L, is 2.37. The recovered unreacted amino acids were always enriched in the L-form. The stereoselective reactivities were influenced by the catalyst concentration and not influenced by the reaction temperature.

In the previous papers, (−)-menthyl esters of α-amino acids have been synthesized from amino acids and (−)-menthol by the use of the azeotropic distillation method[1] and the amino acid *N*-carboxyanhydride (NCA) method.[2] In these reactions, D-form amino acid (−)-menthyl esters gave better yields than L-form esters. This suggests that the reactions proceed through different esterification rates of the two enantiomers.

In the present study, the stereoselective reactivity of amino acid enantiomers for (−)-menthol and the optical purity of the recovered unreacted amino acids were studied. The experimental conditions were as follows. Racemic amino acid NCAs of alanine, valine, and phenylalanine were reacted with one half a molar equivalent of (−)-menthol in dioxane, while the hydrogen chloride concentration and the reaction temperature were varied. After the unreacted NCA has then been hydrolyzed and removed, the amino acid (−)-menthyl esters were acetylated. The amounts of two diastereomers were determined from the gas-liquid-chromatographic (glc) analyses[3] by correcting them with the calibration curves made by authentic amino acid (−)-menthyl esters. From the results obtained under several experimental conditions, the stereoselective reactivities can be said to be influenced by the concentration of hydrogen chloride as a catalyst and not to be influenced by the reaction temperature. We consider that, at high catalyst concentrations, the NCA ring changes to the acid chloride, which easily accepts the steric effect of (−)-menthol.

Experimental

Preparation of Amino Acid (−)-Menthyl Ester Hydrochlorides. The (−)-menthyl ester hydrochlorides of L- and D-alanine, L- and D-valine, and L- and D-phenylalanine were synthesized by azeotropic distillation using *p*-toluenesulfonic acid, as has been described previously.[1]

Acylation of Amino Acid (−)-Menthyl Ester Hydrochlorides. Acetylation: To a solution of L- and D-amino acid (−)-menthyl ester hydrochloride in 10 m*l* of ethyl acetate, a 2 molar equivalent of a 1 N sodium carbonate solution and a 1.5 molar equivalent of acetic anhydride were added at 0°C. After stirring for 1 hr at 0°C and then for 2 hr at room temperature, the organic layer was washed successively with water, a 0.5 N sodium bicarbonate solution, water, 0.5 N hydrochloric acid, and water; dried over sodium sulfate, and evaporated to dryness *in vacuo*. The product was treated with *n*-hexane, filtered, and dried; the yield was, on the average, 90—95%.

Benzoylation: To a solution of L- and D-amino acid (−)-menthyl ester hydrochloride in 5 m*l* of ethanol containing a 2 molar equivalent of sodium ethoxide, a 1.2 molar equivalent of benzoyl chloride was added at 10°C. After stirring for 3 hr, the solution was evaporated to dryness *in vacuo*. The product was extracted with ethyl acetate, and the organic layer was successively washed with water, a 0.5 N sodium bicarbonate solution, water, 0.5 N hydrochloric acid, and water; dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue was treated with *n*-hexane, filtered, and dried; the yield was, on the average, 80—88%.

Retention Times of Standard Samples. The retention times of standard samples, as determined by glc, are shown in Table 1. As is shown in Table 1, the acetyl derivatives were well and rapidly separated. We carried out all the glc analyses using acetyl amino acid (−)-menthyl esters.

TABLE 1. GAS CHROMATOGRAPHIC SEPARATION OF ACYL-α-AMINO ACID MENTHYL ESTERS

Amino acid menthyl ester	Column temp. (°C)	Retention time of diastereomers (min)		t_D/t_L
		t_D[a]	t_L[b]	
Acetyl Ala	175	27	24	1.13
Benzoyl Ala	220	38	33.5	1.13
Acetyl Val	220	15	13	1.15
Benzoyl Val	220	31	27.5	1.13
Acetyl Phe	220	39.5	37	1.07
Benzoyl Phe	220	21.5	19.5	1.10

a) Retention time of acyl-D-amino acid-(−)-menthyl ester.
b) Retention time of acyl-L-amino acid-(−)-menthyl ester.

DL-Amino Acid NCA's. The NCA's were synthesized by the phosgene method.[4] All the NCA's were recrystallized from ethyl acetate and *n*-hexane.

N-Acetyl Amino Acid (−)-Menthyl Esters. To a solution of (−)-menthol (0.01 mol) in 100 m*l* of dioxane containing 1N, 2N, 4N, and 5.8N (saturated) hydrogen chloride as catalysts, DL-amino acid NCA (0.02 mol) was added, then the mixture was reacted with stirring at 10, 20, and 30°C. At regular time intervals, 10-m*l* portions of the reaction mixture were taken out, and then 10 m*l* of water was added to it. The unreacted NCA was hydrolyzed by heating in a boiling-

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[1] K. Harada and T. Hayakawa, This Bulletin, **37**, 191 (1964).
[2] T. Hayakawa and K. Harada, *ibid.*, **38**, 1354 (1965).
[3] B. Halpern and J. W. Westley, *Chem. Commun.*, **1965**, 421.

water bath for 2 min. The dioxane was removed *in vacuo*. The aqueous layer was made alkaline by the addition of a sodium bicarbonate solution. The amino acid (−)-menthyl ester was extracted with 25 m*l* of ethyl acetate. This organic layer was acetylated with acetic anhydride and sodium carbonate in the same way as above. The acetyl amino acid (−)-menthyl ester in ethyl acetate was directly analyzed by glc.

The Test of the Stereoselective Hydrolysis. In order to check the stereoselective hydrolysis of the amino acid menthyl esters in a hydrochloric acid solution in the above experiments, the following experiments were designed. To a solution of DL-alanine menthyl ester (0.0002 mol) in 1.0 m*l* of dioxane, 1.0-m*l* portions of 1N, 2N, 4N, and 6N hydrochloric acid were added separately. Then the mixture was heated in the boiling-water bath for 2 min. After the reaction mixture has been treated in the same way as above, the recovery of the L- and D-alanine menthyl esters was determined by glc. The results are shown in Table 2.

TABLE 2. RECOVERY OF AMINO ACID MENTHYL ESTERS BY HYDROLYSIS

HCl concn. (N)	Recovery, %	
	L-Alanine deriv.	D-Alanine deriv.
1	100.0	100.0
2	98.8	100.0
4	92.4	94.1
6	88.7	90.6

As is shown in Table 2, the hydrolysis of the ester was little affected by heating in 1N and 2N hydrochloric acid. In higher concentrations of hydrochloric acid, the stereoselective hydrolysis was observed slightly in two diastereomers. The recovery ratio of the two diastereomers (D/L) was 1.02 in 6N hydrochloric acid. This ratio suggests that the stereoselective hydrolysis has little influence on the stereoselective reactivity of DL-NCA for (−)-menthol.

Recovery of Unreacted Amino Acid. Amino acid NCA (0.002 mol) and a one-half molar equivalent of (−)-menthol (0.001 mol) in 10 m*l* of 4N HCl/dioxane were reacted at 20°C for 12 hr. After the same treatment as has been described above, the aqueous layer was made alkaline by sodium bicarbonate. The amino acid (—)-menthyl ester was extracted with ethyl acetate. To this basic aqueous solution, 2,4-dinitrofluorobenzene (DNFB, 0.0018 mol) was added, and the mixture was stirred at 40°C until a clear solution was obtained. The unreacted DNFB was extracted with ether. The aqueous layer was acidified to Congo Red with 6N hydrochloric acid and extracted with 50 m*l* of ether. The ethereal layer was washed with water, dried over sodium sulfate, and then passed through a cellite column treated with a pH-6.5 phosphate buffer[5] to purify it. The organic solvent was evaporated to dryness *in vacuo*, and the residue was redissolved in 1N sodium hydroxide. From the absorbance of the ultraviolet absorption at nearly 360 mμ of this solution, the yields of DNP-amino acids were calculated. The yields and $[\alpha]_D$ are shown in Table 3. From the $[\alpha]_D$ values of the DNP-amino acids shown in Table 3, each amino acid is L-form rich. This suggests that the (−)-menthyl esterification of DL-amino acid prefers the D-form to the L-form.

TABLE 3. RECOVERY OF UNREACTED α-AMINO ACID

DNP-Amino acid	Recovery[a) %]	$[\alpha]_D$ 1N NaOH	Confign.	Optical purity,[b)] %
Ala	41.0	+8.6°	L	5.9
Val	26.1	+4.2°	L	3.9
Phe	49.1	−3.8°	L	4.1

a) Recoveries were calculated from starting DL-amino acid NCA. b) Defined as ($[\alpha]_D$ obsd/$[\alpha]_D$ lit)×100. DNP-L-Ala, $[\alpha]_D$ +143.9° (1N NaOH); DNP-L-Val, $[\alpha]_D$ +109.2° (1N NaOH); DNP-L-Phe, $[\alpha]_D$ −93.6° (1 N NaOH).

Method. Glc analyses were carried out on a Hitachi K 53 gas chromatograph with a flame ionization detector, using a 1 m×3 mm glass column (60/80 mesh 20% poly-neopenthyl glycol succinate on chromosorb W AW DMCS) and with a nitrogen flow rate of 30 m*l*/min.

Results and Discussion

The Reaction Rates and Their Ratios of the Esterification of Two Enantiomers. The yields of the diastereomers obtained by the reaction of DL-alanine NCA and (−)-menthol at 20°C using 4 N HCl/dioxane are shown in Fig. 1 as a function of the time. This suggests that the esterification rate of D-amino acid, V_D, is larger than that of L-amino acids, V_L. At their initial stages, a linear relationship exists between the yields of the amino acid (−)-menthyl esters and the reaction time. At a prolonged reaction time, though the increments of both yields per unit of time decreased, constant (V_D/V_L) ratios were observed. The dependences on the temperature and the concentration of the hydrogen chloride catalyst for stereoselective reactivity of each amino acid NCA are shown in Table 4. From these results, the ratios of the reactivity of the diastereomers were calculated. As is shown in Table 4, V_D and V_L increase with an increase in the temperature and in the concentration of a catalyst under each set of conditions. In all cases, V_D is larger than V_L. This suggests that the ratio of stereoselective reactivity, V_D/V_L, is not influenced by the reaction temperature, but is influenced by the concentration, of a catalyst.

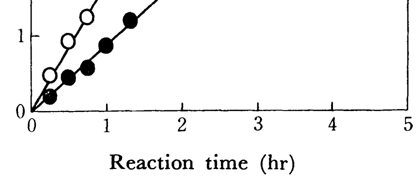

Fig. 1. The relationship between the reaction time and the yield of D- and L-alanine (−)-menthyl esters.
○: D-alanine (−)-menthyl ester,
●: L-alanine (−)-menthyl ester.

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[4] A. C. Farthing, *J. Chem. Soc.*, **1950**, 3213.

[5] A. J. Woiwod, *J. Gen. Microbiol.*, **3**, 312 (1949).

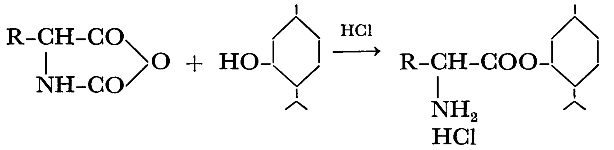
TABLE 4. REACTION RATES AND RATIOS OF DL-AMINO ACID NCAS FOR (—)-MENTHOL

DL-Amino acid NCA	HCl concn. (N)	Reaction rates and ratios								
		At 10°C			At 20°C			At 30°C		
		V_{D}a)	V_{L}b)	$V_{\mathrm{D}}/V_{\mathrm{L}}$	V_{D}	V_{L}	$V_{\mathrm{D}}/V_{\mathrm{L}}$	V_{D}	V_{L}	$V_{\mathrm{D}}/V_{\mathrm{L}}$
Ala	1	0.051	0.046	1.10	0.058	0.052	1.12	0.071	0.062	1.15
	2	0.21	0.15	1.40	0.44	0.32	1.38	1.09	0.79	1.38
	4	0.34	0.17	2.00	1.70	0.88	1.93	1.82	0.96	1.90
	5.8	0.77	0.36	2.14						
Val	1	0.0032	0.0029	1.10	0.0066	0.0057	1.16	0.0097	0.0083	1.17
	2	0.0083	0.0065	1.28	0.020	0.016	1.25	0.10	0.075	1.33
	4	0.070	0.037	1.89	0.13	0.068	1.87	0.21	0.11	1.91
	5.8	0.20	0.084	2.37						
Phe	1	0.022	0.021	1.05	0.041	0.038	1.09	0.078	0.072	1.08
	2	0.045	0.035	1.29	0.059	0.045	1.31	0.098	0.075	1.31
	4	0.39	0.28	1.39	0.53	0.38	1.39	0.62	0.45	1.38
	5.8	0.55	0.32	1.72						

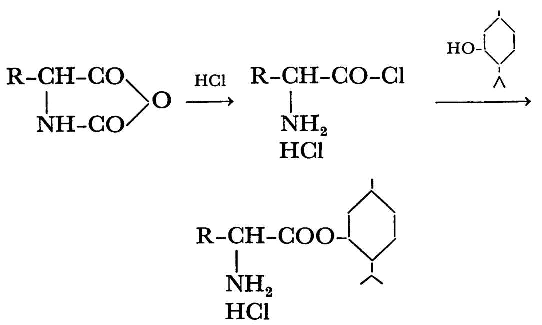
a) Reaction rate of D-amino acid NCA (mg/m*l*·hr).
b) Reaction rate of L-amino acid NCA (mg/m*l*·hr).

Moreover, the maximum ratios of stereoselectivity were observed when the reaction was carried out using saturated hydrogen chloride as a catalyst in three amino acids. As is shown in Table 3, the recovered unreacted amino acids are always L-form rich.

Reaction Mechanism. It was found that the stereoselective reactivity of DL-amino acid NCA for (—)-menthol is not influenced by the reaction temperature in the range from 10 to 30°C, but is influenced by the catalyst concentration of hydrogen chloride. This suggests that the reaction may proceed by the route of scheme 1 or 2.



(1)



(2)

At low hydrogen chloride concentrations, the reaction may proceed mainly by the route of scheme 1 and the stereoselective reactivity is small. On the other hand, at high hydrogen chloride concentrations, the reaction may proceed by the route of scheme 2 and large stereoselectivities are obtained.

TABLE 5. REACTION RATES AND RATIOS FOR (—)-MENTHOL AFTER REACTED WITH DL-VALINE NCA AND HYDROGEN CHLORIDE

Reaction time (hr)	V_{D}a)	V_{L}b)	$V_{\mathrm{D}}/V_{\mathrm{L}}$
0	0.025	0.021	1.19
1	0.035	0.027	1.30
2	0.041	0.029	1.41
3	0.044	0.030	1.47

Solvent: 2N HCl/dioxane, Temp: 20°C
a) Reaction rate of D-amino acid NCA (mg/m*l*·hr).
b) Reaction rate of L-amino acid NCA (mg/m*l*·hr).

In order for the reaction to proceed *via* the route of scheme 2 exclusively, DL-valine NCA was reacted with 2 N HCl/dioxane for 1, 2, and 3 hr at 20°C, and then (—)-menthol was added to the solution. This mixture was allowed to react for 2.5 hr. The valine (—)-menthyl ester thus obtained was analyzed by glc in order to determine the yield of each diastereomer by the method described above. The results are shown in Table 5. As is shown in Table 5, the higher stereoselectivity was observed when the NCA and hydrogen chloride were kept for a long time before the addition of (—)-menthol. Therefore, it is considered that, at high hydrogen chloride concentrations, the NCA changes to the acid chloride, which is more susceptible to the steric effect of (—)-menthol. Such a difference may cause the higher stereoselective reactivity.

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