

189. Yukio Kimura^{*1} : Studies on Acylase Activity and Micro-organisms. XXI.^{*2} Optical Resolution of Higher Amino Acids by Acylase of Soil Bacteria.

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In 1923, Abderhalden¹⁾ discussed the resolution of N-formyl-DL-2-aminooctanoic acid through the chemical procedure. Thereafter, Baker and Meister²⁾ reported that N-chloroacetyl-2-aminoheptanoic acid, 2-aminooctanoic amide, and 2-aminolauric amide were asymmetrically hydrolyzed by the hog kidney. Subsequently, Greenstein, *et al.*³⁾ confirmed that N-chloroacetyl derivatives of normal 2-amino acids, from 2-aminoheptanoic to 2-aminoundecylic, were asymmetrically hydrolyzed by renal acylase I.

Since 1950, the optical resolution of amino acids by soil bacteria have been studied in this laboratory. In this paper, the optical resolution of higher 2-amino acid by acylase of a strain (KT 218)⁴⁾ of soil bacteria isolated in this laboratory is described. Incidentally, KT 218 could hydrolyze asymmetrically N-benzoyl and N-acetyl derivatives of threonine, lysine, tryptophan, and methionine. At the outset, N-benzoyl and N-dichloroacetyl derivatives of 2-aminooctanoic, 2-aminodecanoic, 2-aminolauric, 2-amino-

TABLE I. Acylated Higher 2-Amino Acids

No.	Compound	m.p. (°C)	Yield (%)	mol. Formula	Analysis (%)					
					Calcd.			Found		
					C	H	N	C	H	N
(I)	N-Dichloroacetyl-DL-2-aminooctanoic acid ^{a)}	118~119	75	C ₁₀ H ₁₇ O ₃ NCI ₂	44.41	6.29	5.17	44.56	6.17	5.13
(II)	N-Benzoyl-DL-2-aminooctanoic acid ^{a)}	127	80	C ₁₆ H ₂₁ O ₃ N	68.41	8.04	5.32	68.62	8.15	5.14
(III)	N-Dichloroacetyl-DL-2-aminodecanoic acid ^{b)}	105~106	73	C ₁₂ H ₂₁ O ₃ NCI ₂	48.27	7.03	4.69	48.41	7.20	4.81
(IV)	N-Benzoyl-DL-2-aminodecanoic acid ^{b)}	126~128	82	C ₁₇ H ₂₅ O ₃ N	70.07	8.06	4.81	70.21	8.21	4.91
(V)	N-Dichloroacetyl-DL-2-aminolauric acid ^{b)}	100~101	68	C ₁₄ H ₂₅ O ₃ NCI ₂	51.57	7.65	4.28	51.70	7.81	4.41
(VI)	N-Benzoyl-DL-2-aminolauric acid ^{b)}	118~120	78	C ₁₉ H ₂₉ O ₃ N	71.44	9.15	4.39	71.68	9.40	4.31
(VII)	N-Dichloroacetyl-DL-2-aminomyristic acid ^{b)}	100~101	33	C ₁₆ H ₂₉ O ₃ NCI ₂	54.17	8.20	3.95	54.47	8.38	4.20
(VIII)	N-Benzoyl-DL-2-aminomyristic acid ^{b)}	119~120	76	C ₂₁ H ₃₃ O ₃ N	72.58	9.57	4.03	72.63	9.70	4.15
(IX)	N-Dichloroacetyl-DL-2-aminopalmitic acid ^{b)}	99~100	31	C ₁₈ H ₃₃ O ₃ NCI ₂	56.48	8.26	3.66	56.79	8.30	3.79
(X)	N-Benzoyl-DL-2-aminopalmitic acid ^{b)}	113~115	72	C ₂₃ H ₃₇ O ₃ N	73.58	9.93	3.73	73.71	10.06	3.86

a) Recrystallized from AcOEt

b) Recrystallized from Me₂CO and petr. ether. All compounds are tabular crystals

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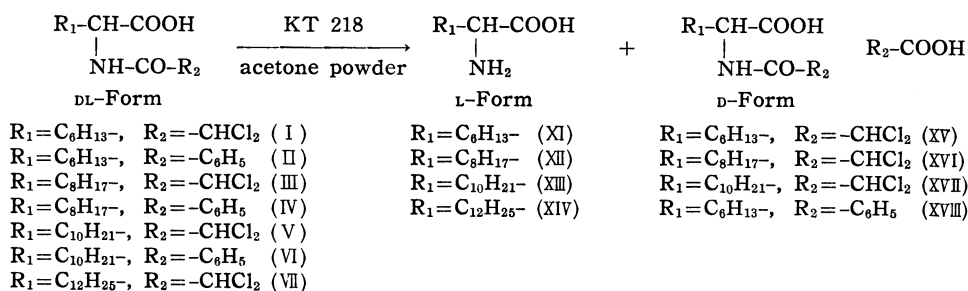
myristic, and 2-aminopalmitic acids were synthesized (Table I). The acylase activity of KT 218 was tested on 10 acylamino acids mentioned above by means of Grassmann and Heyde's ethanolic titration method⁵⁾ (Table II).

TABLE II. Acylase Activity of KT 218 Acetone Powder

Substrates	Activity ^{a)}
N-Dichloroacetyl-DL-2-aminooctanoic acid (I)	22
N-Benzoyl-DL-2-aminooctanoic acid (II)	14
N-Dichloroacetyl-DL-2-aminodecanoic acid (III)	6
N-Benzoyl-DL-2-aminodecanoic acid (IV)	4
N-Dichloroacetyl-DL-2-aminolauric acid (V)	5
N-Benzoyl-DL-2-aminolauric acid (VI)	3
N-Dichloroacetyl-DL-2-aminomyristic acid (VII)	2
N-Benzoyl-DL-2-aminomyristic acid (VIII)	0
N-Dichloroacetyl-DL-2-aminopalmitic acid (IX)	0
N-Benzoyl-DL-2-aminopalmitic acid (X)	0

^{a)} Expressed as μ mole hydrolyzed/hr./10 mg. of the acetone powder
The digest consisted of 10 mg. of the acetone powder, 2 cc. of H₂O, and 2 cc. of 0.05M neutralized substrates (pH 8.0) at 37°.

From Table II, it was shown that the dichloroacetyl amino acids were more easily hydrolyzed than the corresponding benzoylamino acids. The ability of KT 218 to resolve dichloroacetyl-DL-2-amino acids were tested as follows: To 0.05M of dichloroacetyl-DL-2-amino acid solution (pH 8.0) the bacterial acetone powder was added and the mixture was incubated at 37° with a few drops of toluene for 2~5 days. N-Dichloroacetyl-DL-2-aminooctanoic acid (I), N-dichloroacetyl-DL-2-aminodecanoic acid (III), and N-dichloroacetyl-DL-2-aminolauric acid (V) produced L-2-aminooctanoic acid (XI) and N-dichloroacetyl-D-2-aminooctanoic acid (XV), L-2-aminodecanoic acid (XII) and N-dichloroacetyl-D-2-aminodecanoic acid (XVI), and L-2-aminolauric acid (XIII) and N-dichloroacetyl-D-2-aminolauric acid (XVII) in good yields, respectively. But N-Dichloroacetyl-DL-2-aminomyristic acid (VII) gave L-2-aminomyristic acid (XIV) and impure N-dichloroacetyl-D-2-aminomyristic acid, and then N-dichloroacetyl-DL-2-aminopalmitic acid (IX) could not be hydrolyzed. The free L-amino acids, (XI), (XII), and (XIII) were led to their N-dichloroacetyl derivatives, respectively.



Subsequently, KT 218 could hydrolyze asymmetrically N-benzoyl-DL-2-aminooctanoic acid (II) to yield L-2-aminooctanoic acid (XI) and N-benzoyl-D-2-aminooctanoic acid (XVIII) in good yields. But, N-benzoyl-DL-2-aminodecanoic acid (IV) and N-benzoyl-DL-2-aminolauric acid (VI) gave L-2-aminodecanoic acid (XII) and impure N-benzoyl-D-2-aminodecanoic acid and L-2-aminolauric acid (XIII) and impure N-benzoyl-D-2-aminolauric acid, respectively. N-Benzoyl-DL-2-aminomyristic acid (VIII) and N-benzoyl-DL-2-aminopalmitic acid (X) could not be hydrolyzed.

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Experimental

Preparation of Higher 2-Amino Acids—The free fatty acids, from octanoic to palmitic acid, were Nippon Yushi products. Each was derived to 2-amino octanoic, 2-aminodecanoic, 2-aminolauric, 2-aminomyristic and 2-aminopalmitic acid according to the method described by Greenstein, *et al.*³⁾

N-Dichloroacetyl and N-Benzoyl Derivatives of Higher 2-Amino Acids—(I), (II), (III), (IV), (V), (VI), (VIII) and (X) were prepared according to Shotten Baumann's method and (VII) and (IX) were prepared by warming the mixture of free 2-amino acid, dichloroacetic anhydride and dichloroacetic acid.

Growth Condition and Preparation of Acetone Powder of KT 218—KT 218 was grown in five 1 L. Erlenmeyer flasks each containing 200 cc. of bouillon (pH 7.2). Growth was allowed to proceed at 25° for 3 days, then the cells were harvested by centrifugation, and washed with dist. H₂O. The cells thus obtained were placed in 300 cc. of Me₂CO cooled to -5° and stirred vigorously for 5 min. The resulting solid was collected by suction and washed first with cold Me₂CO and then with cold Et₂O. The filter cake of cells was transferred onto a sheet of paper and worked gently with a spatula until the solvent evaporated, leaving a dry powder which weighted approx. 0.4 g.

Asymmetric Hydrolysis of N-Dichloroacetyl-DL-2-aminooctanoic Acid (I)—A suspension of 2.7 g. of (I) in 200 cc. of H₂O was brought into solution at pH 8.0 by the addition of 10% NaOH. To this aqueous solution, 0.4 g. of KT 218 acetone powder was added, and allowed to digest at 37° with 2 cc. of toluene. After 2 days, the crystals of L-2-aminooctanoic acid (XI) separated during the incubation were collected by centrifugation. The clear supernatant was adjusted at pH 4.5 by the addition of AcOH and to this an equal volume of EtOH was added. The precipitate was removed and the filtrate was concentrated *in vacuo* until crystallization began. EtOH was then added and allowed to stand overnight in a refrigerator. Thus precipitated L-2-aminooctanoic acid (XI) was also collected. Recrystallization of the combined precipitate of (XI) from 50% EtOH yielded 0.62 g. (78%) as tabular crystals, $[\alpha]_D^{16} + 24^\circ$ (c=1, 5N HCl). *Anal.* Calcd. for C₈H₁₇O₂N: C, 60.34; H, 10.76; N, 8.80. Found: C, 60.48; H, 10.81; N, 8.71.

The filtrate from (XI) was concentrated to dryness and the residue was dissolved in a small amount of H₂O. The solution was acidified with HCl, extracted with Et₂O, and Et₂O was evaporated to give a syrupy residue of N-dichloroacetyl-D-2-aminooctanoic acid (XV) which gradually crystallized by the addition of petr. ether. Recrystallization from AcOEt and petr. ether yielded 1.0 g. (74%) of (XV) as needles, m.p. 93°, $[\alpha]_D^{16} - 8.3^\circ$ (c=2, Me₂CO). *Anal.* Calcd. for C₁₀H₁₇O₃NCl₂: C, 44.41; H, 6.29; N, 5.17. Found: C, 44.40; H, 6.35; N, 5.34.

(XI) was characterized as its dichloroacetate (XV-L), m.p. 93°, $[\alpha]_D^{16} + 8.2^\circ$ (c=2, Me₂CO). *Anal.* Calcd. for C₁₀H₁₇O₃NCl₂: C, 44.41; H, 6.29; N, 5.17. Found: C, 44.53; H, 6.36; N, 5.23. The mixture of (XV) and (XV-L) melted at 115° (N-dichloroacetyl-DL-2-aminooctanoic acid melts at 118~119).

Asymmetric Hydrolysis of N-Dichloroacetyl-DL-2-aminodecanoic Acid (III)—To 200 cc. of 0.05M (III) solution, 0.8 g. of KT 218 acetone powder was added and the mixture was incubated at 37° for 5 days affording 0.6 g. (64%) of L-2-aminodecanoic acid (XII), as tabular crystals, which on recrystallization from N HCl had, $[\alpha]_D^{16} + 24.0^\circ$ (c=2, CHCl₃COOH). *Anal.* Calcd. for C₁₀H₂₁O₂N: C, 64.13; H, 11.30; N, 7.48. Found: C, 64.28; H, 11.48; N, 7.51, and 1.04 g. (72%) of N-dichloroacetyl-D-2-aminodecanoic acid (XVI) as tabular crystals, which on recrystallization from Me₂CO and petr. ether had, m.p. 80~82°, $[\alpha]_D^{16} - 7.2^\circ$ (c=2, Me₂CO). *Anal.* Calcd. for C₁₂H₂₁O₃NCl₂: C, 48.27; H, 7.03; N, 4.69. Found: C, 48.52; H, 7.21; N, 4.58. (XII) was characterized as its dichloroacetate (XVI-L), m.p. 80~82°, $[\alpha]_D^{16} + 7.0^\circ$ (c=2, Me₂CO). *Anal.* Calcd. for C₁₂H₂₁O₃NCl₂: C, 48.27; H, 7.03; N, 4.69. Found: C, 48.56; H, 7.21; N, 4.91. The mixture of (XVI) and (XVI-L) melted at 100° (N-dichloroacetyl-DL-2-aminodecanoic acid melts at 105~106°).

Asymmetric Hydrolysis of N-Dichloroacetyl-DL-2-aminolauric Acid (V)—To 200 cc. of 0.05M (V) solution, 0.8 g. of KT 218 acetone powder was incubated at 37° for 5 days affording 0.67 g. (62%) of L-2-aminolauric acid (XIII), as tabular crystals, which on recrystallization from N HCl had, $[\alpha]_D^{15} + 20.5^\circ$ (c=2, CHCl₃COOH). *Anal.* Calcd. for C₁₂H₂₅O₂N: C, 66.93; H, 11.70; N, 6.51. Found: C, 67.03; H, 11.63; N, 6.54., and 1.06 g. (65%) of N-dichloroacetyl-D-2-aminolauric acid (XVII), as tabular crystals, which on recrystallization from Me₂CO and petr. ether had, m.p. 66~68°, $[\alpha]_D^{15} - 6.6^\circ$ (c=2, Me₂CO). *Anal.* Calcd. for C₁₄H₂₅O₃NCl₂: C, 51.47; H, 7.65; N, 4.28. Found: C, 51.68; H, 7.88; N, 4.38.

(XIII) was characterized as its dichloroacetate (XVII-L), m.p. 67~69°, $[\alpha]_D^{15} + 6.8^\circ$ (c=2, Me₂CO). *Anal.* Calcd. for C₁₄H₂₅O₃NCl₂: C, 51.47; H, 7.65; N, 4.28. Found: C, 51.63; H, 7.78; N, 4.40. The mixture of (XVII) and (XVII-L) melted at 95° (N-dichloroacetyl-DL-2-aminolauric acid melts at 100~101°).

Asymmetric Hydrolysis of N-Dichloroacetyl-DL-2-aminomyristic Acid (VII)—To 200 cc. of 0.05M (VII) solution, 0.8 g. of KT 218 acetone powder was added and the mixture was incubated at 37° for

6 days affording 0.23 g. (19%) of L-2-aminomyristic acid (XIV), as tabular crystals, which on recrystallization from *N* HCl had, $[\alpha]_D^{13} + 12.5^\circ$ ($c=2$, CHCl_3COOH). *Anal.* Calcd. for $\text{C}_{14}\text{H}_{29}\text{O}_2\text{N}$: C, 69.09; H, 12.01; N, 5.76. Found: C, 68.87; H, 12.32; N, 5.80., and impure N-dichloroacetyl-D-2-aminomyristic acid.

Asymmetric Hydrolysis of N-Benzoyl-DL-2-aminooctanoic Acid (II)—A suspension of 2.63 g. of (II) in 200 cc. of H_2O was brought into solution at pH 8.0 by the addition of 10% NaOH. To this aqueous solution, 0.4 g. of KT 218 acetone powder was added, and allowed to digest at 37° with 2 cc. of toluene. After 4 days the crystals of L-2-aminooctanoic acid (XI) separated during the incubation were collected by centrifugation. Recrystallization from 50% EtOH yielded 0.6 g. (75.5%) of (XI) as tabular crystals, $[\alpha]_D^{12} + 24.0^\circ$ ($c=1$, 5*N* HCl). *Anal.* Calcd. for $\text{C}_8\text{H}_{17}\text{O}_2\text{N}$: C, 60.34; H, 10.76; N, 8.80. Found: C, 60.54; H, 10.69; N, 8.67.

After the removal of (XI) from the digestion mixture, the filtrate was concentrated *in vacuo*, acidified with HCl, and the resulting precipitate was collected by filtration. The product was washed several times with petr. ether to remove BzOH, and recrystallized from AcOEt and petr. ether to give 1.0 g. (76%) of (XVIII) as tabular crystals, m.p. $71\sim 73^\circ$, $[\alpha]_D^{12} - 9.0^\circ$ ($c=2$, Me_2CO). *Anal.* Calcd. for $\text{C}_{15}\text{H}_{21}\text{O}_3\text{N}$: C, 68.41; H, 8.04; N, 5.32. Found: C, 68.37; H, 7.97; N, 5.41. Evaporation of petr. ether yielded 0.5 g. (82%) of BzOH, m.p. 118° which showed no depression with the authentic sample.

(XI) was characterized as its benzoate (XVIII-L), m.p. $72\sim 74^\circ$, $[\alpha]_D^{13} + 8.5^\circ$ ($c=2$, Me_2CO). *Anal.* Calcd. for $\text{C}_{15}\text{H}_{21}\text{O}_3\text{N}$: C, 68.41; H, 8.04; N, 5.82. Found: C, 68.63; H, 7.92; N, 5.38. The mixture of (XVIII) and (XVIII-L) melted at 120° (N-benzoyl-DL-2-aminooctanoic acid melts at 127°).

Asymmetric Hydrolysis of N-Benzoyl-DL-2-aminodecanoic Acid (IV)—To 200 cc. of 0.05*M* (IV) solution, 0.8 g. of KT 218 acetone powder was added and the mixture was incubated at 37° for 6 days affording 0.41 g. (44%) of L-2-aminodecanoic acid (XIII), recrystallized from *N* HCl, $[\alpha]_D^{13} + 24.0^\circ$ ($c=2$, CHCl_3COOH). *Anal.* Calcd. for $\text{C}_{10}\text{H}_{21}\text{O}_2\text{N}$: C, 64.13; H, 11.30; N, 7.48. Found: C, 64.01; H, 11.41; N, 7.60., 0.28 g. (46%) of BzOH and impure N-benzoyl-D-2-aminodecanoic acid.

Asymmetric Hydrolysis of N-Benzoyl-DL-2-aminolauric Acid (VI)—To 200 cc. of 0.05*M* (VI) solution, 0.8 g. of KT 218 acetone powder was added and the mixture was incubated at 37° for 6 days affording 0.42 g. (39%) of L-2-aminolauric acid (XIII), recrystallized from *N* HCl, $[\alpha]_D^{15} + 20.5^\circ$ ($c=2$, CHCl_3COOH). *Anal.* Calcd. for $\text{C}_{12}\text{H}_{25}\text{O}_2\text{N}$: C, 66.93; H, 11.70; N, 6.51. Found: C, 66.71; H, 11.52; N, 6.40., 0.25 g. (41%) of BzOH and impure N-benzoyl-D-2-aminolauric acid.

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Summary

The acylase activity of a strain (KT 218) of soil bacteria was tested against N-benzoyl and N-dichloroacetyl derivatives of 2-aminooctanoic, 2-aminodecanoic, 2-aminolauric, 2-aminomyristic, and 2-aminopalmitic acid. It was revealed that the dichloroacetyl amino acids were more easily hydrolyzed than the corresponding benzoyl amino acids and that the N-dichloroacetyl-derivatives of the homologous series of straight chain 2-amino acid were hydrolyzed at progressively decreasing rates from N-dichloroacetyl-DL-2-aminooctanoic acid to N-dichloroacetyl-DL-2-aminopalmitic acid which was resistant to KT 218 acylase.

The acetone powder of KT 218 effected asymmetric hydrolysis of N-dichloroacetyl derivatives of 2-aminooctanoic, 2-aminodecanoic, 2-aminolauric, 2-aminomyristic acids to yield respectively L-2-aminooctanoic acid (XI) and N-dichloroacetyl-D-2-aminooctanoic acid (XV), L-2-aminodecanoic acid (XIII) and N-dichloroacetyl-D-2-aminodecanoic acid (XVI), L-2-aminolauric acid (XIII) and N-dichloroacetyl-D-2-aminolauric acid (XVII), and L-2-aminomyristic acid (XIV) and impure N-dichloroacetyl-D-2-aminomyristic acid. In the same way KT 218 acetone powder effected asymmetric hydrolysis of N-benzoyl derivatives of 2-aminooctanoic, 2-aminodecanoic, and 2-aminolauric acids to yield respectively L-2-aminooctanoic acid (XI) and N-benzoyl-D-2-aminooctanoic acid (XVIII), L-2-aminodecanoic acid (XIII) and impure N-benzoyl-D-2-aminodecanoic acid, and L-2-aminolauric acid (XIII) and impure N-benzoyl-D-2-aminolauric acid.

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