The Synthesis of α-Amino Acid Menthyl Esters. II[1]

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In the previous study,[2] menthyl esters of α-amino acids have been synthesized from amino acids and menthol by the use of the azeotropic distillation method. However, the reaction conditions of this method were so strong as to decompose serine during the reaction. Some amino acids, such as optically active α-phenylglycine, one of the most racemizable amino acids, may also lose their optical activity during the reaction. In order to use these menthyl esters of α-amino acids as stereochemical standard compounds, it is necessary to compare them with α-amino acid menthyl esters which have been synthesized under milder reaction conditions.

1) Contribution No. 43 of the Institute of Molecular Evolution, University of Miami.

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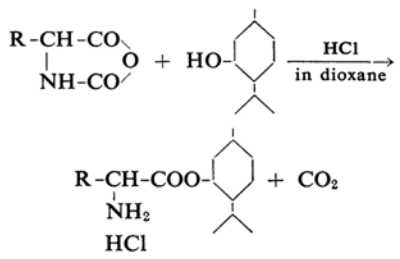
2) K. Harada and T. Hayakawa, This Bulletin, 37, 191 (1964).

α-Amino acid *N*-carboxy anhydride (α-amino acid NCA) has been used in the synthesis of amino acid benzyl esters by Bergmann et al.[3] and later by Erlanger and Brand[4].

In this report, the synthesis of α-amino acid *l*-menthyl esters, under mild conditions, by the use of α-amino acid NCA and *l*-menthol will be described. α-Amino acid NCA prepared by the phosgene method[5] was dissolved in dry dioxane or in a dioxane-ether mixture



containing *l*-menthol ($[\alpha]_D$ −50.0° (*c* 3.50, abs. EtOH), m. p. 42—44°C) and hydrogen chloride. The reaction mixtures were then allowed to stand for one to four days at room temperature. The α-amino acid menthyl esters were isolated as the hydrochlorides; their names will be abbreviated as amino acid M·HCl. The fact that the amino acid M·HCl synthesized under these milder reaction conditions exhibited physical properties almost identical to those observed in the earlier study[2] suggests that the racemization of the amino acid M·HCl prepared by the earlier azeotropic distillation method[2] is small. In this method and also in the earlier method,[2] higher yields of D-amino acid M·HCl than of L-amino acid M·HCl were observed. The fractionation of two diastereomers of the racemic amino acid M·HCl was also observed. The specific rotations of the isolated DL-phenylalanine M·HCl before and after recrystallization were −56.0° and −65.0° respectively, whereas the calculated value is −47.8°.

One advantage of this method is that it can be used for the synthesis of L- and DL-serine M·HCl from *O*-benzyl L- and DL-serine NCA and menthol. *O*-Benzyl-L-serine[6] and *O*-benzyl-DL-serine[7] were treated with phosgene to convert them to the corresponding *O*-benzyl L-serine NCA and *O*-benzyl DL-serine NCA. These NCAs were reacted with *l*-menthol in dry dioxane and ether in the presence of hydrogen chloride at room temperature for four days. *O*-Benzyl L-serine and DL-serine menthyl-esters were thus isolated as hydrochlorides. The attempted debenzylation of these *O*-benzyl L- and DL-serine menthyl-esters by acid hydrolysis (5 N hydrochloric acid, boiling for one hour) was not successful, whereas *O*-benzyl serine was easily debenzylated by acid hydrolysis.[6,7] The debenzylation of α-amino acid M·HCl was carried out under relatively strong hydrogenolysis conditions through the use of 5% palladium on charcoal.

The specific rotations of *O*-benzyl L-ser M·HCl and *O*-benzyl DL-ser M·HCl were found to be −47.5° and −46.5° respectively. However, after debenzylation, L-ser M·HCl and DL-ser M·HCl showed specific rotations of −69.8° and −56.6° respectively. The fractionation of *O*-benzyl DL-ser M·HCl by the recrystallization procedure (ethyl acetate - ether) was also observed. Three fractions of *O*-benzyl DL-ser M·HCl were isolated (see Experimental section): Fractions I (m. p. 179—180°C), II (m. p. 135—150°C) and III (m. p. 128—130°C). The melting point of fraction I (the least soluble fraction) rose to 181—182°C after recrystallization. Fraction III (the most soluble fraction) has the same melting point and optical rotation as *O*-benzyl-L-ser M·HCl. The mixed melting point test of fraction III with *O*-benzyl-L-ser M·HCl showed no depression of the melting point. The hydrogenolysis of fraction III gave serine M·HCl, which showed $[\alpha]_D^{17}$ −67.8° and a m. p. of 218—219°C. The mixed melting point test of serine M·HCl obtained from fraction III and L-serine M·HCl indicated that the two compounds were identical. These results show that *O*-benzyl DL-serine M·HCl is considerably fractionated by the recrystallization procedure to the least soluble fraction (*O*-benzyl-D-ser M·HCl rich) and the most soluble fraction (*O*-benzyl-L-ser M·HCl rich). DL-Serine M·HCl was also prepared from *O*-acetyl DL-ser NCA and *l*-menthol under similar reaction conditions. The *O*-acetyl group was removed during the reaction. The specific rotation of the isolated DL-serine M·HCl was −56.9° before recrystallization. After recrystallization, two fractions of DL-serine M·HCl, I and II, were isolated. Fraction I decomposed at 213—220°C and showed a specific rotation of $[\alpha]_D^{26}$ −61.6°. Fraction II, which was less alcohol-soluble, decomposed at 209—214°C and showed a specific rotation of $[\alpha]_D^{26}$ −68.7°. Elemental analyses of both I and II agreed with the theoretical value of serine M·HCl. Fractions I and II are both supposed to be L-rich fractions, while fraction II might be an almost pure L-ser M·HCl.

3) M. Bergmann, L. Zervas and W. F. Ross, *J. Biol. Chem.*, **111**, 245 (1933).
4) B. F. Erlanger and E. Brand, *J. Am. Chem. Soc.*, **73**, 3508, 4025 (1951).
5) A. C. Farthying and R. J. W. Reynolds, *Nature*, **165**, 647 (1950); A. C. Farthying, *J. Chem. Soc.*, **1951**, 2294.
6) K. Okawa, This Bulletin, **29**, 486 (1956).
7) K. Okawa and H. Tani, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **75**, 1199 (1954).

TABLE I. α-AMINO ACID MENTHYL ESTER HYDROCHLORIDES, R-CH-COOM
NH₂ HCl

α-Amino acid M·HCl	Yield %	Recryst. solvent[a]	After recryst. m. p. °C[b]	After recryst. $-[\alpha]_D^{25-26}$ d)	c	Molecular formula	Elemental analysis Calcd., % C	H	N	Found, % C	H	N
D-Ala	75	Et	188—189	72.0	1.74	$C_{13}H_{26}O_2NCl$	59.18	9.93	5.31	59.25	10.10	5.21
L-Ala	74	Et	219—220	66.7	1.89	$C_{13}H_{26}O_2NCl$	59.18	9.93	5.31	59.14	9.83	5.20
D-Phe	63	Et	187—188	77.8	1.57	$C_{19}H_{30}O_2NCl$	67.14	8.90	4.12	67.19	8.98	4.05
L-Phe	49	Et	162—163	13.8	3.23	$C_{19}H_{30}O_2NCl$	67.14	8.90	4.12	67.22	9.09	3.94
DL-Phe	73	Et	145—169[c]	65.0	1.77	$C_{19}H_{30}O_2NCl$	67.14	8.90	4.12	66.96	8.91	4.27
D-Val	73	Et	207—208	74.4	1.69	$C_{15}H_{30}O_2NCl$	61.71	10.36	4.80	61.85	10.60	4.85
L-Val	47	Et	225—226	49.8	2.64	$C_{15}H_{30}O_2NCl$	61.71	10.36	4.80	61.66	10.37	4.77
D-Phenylgly	84	A	253—254	127.0	1.03	$C_{18}H_{28}O_2NCl$	66.34	8.66	4.30	66.41	8.68	4.38
L-Phenylgly	82	A	253—254	− 9.6	0.98	$C_{18}H_{28}O_2NCl$	66.34	8.66	4.30	66.42	8.63	4.57
DL-Phenylgly	86	A	252—253	65.7	1.10	$C_{18}H_{28}O_2NCl$	66.34	8.66	4.30	66.35	8.57	4.39
DL-Ser[e]	68	A	(I) 213—220 decomp.[c] (II) 209—214 decomp.[c]	(I) 61.6 (II) 68.7	1.65 1.16	$C_{13}H_{26}O_3NCl$	55.80	9.37	5.01	(I) 55.83 (II) 55.85	9.42 9.56	5.00 5.13
O-Benzyl L-Ser	68	EP	132—133	47.5[h]	1.11	$C_{20}H_{32}O_3NCl$	64.94	8.72	3.73	64.75	8.68	3.68
O-Benzyl DL-Ser	68	Et	(I) 181—182	46.0[h]	1.13	$C_{20}H_{32}O_3NCl$	64.94	8.72	3.73	65.18	8.67	3.65
L-Ser[f]	66	A	216—219 decomp.	69.8[h]	0.702	$C_{13}H_{26}O_3NCl$	55.80	9.37	5.01	55.75	9.29	5.08
DL-Ser[g]	90 53	A A	(I) 164—166 (II) 218—219 decomp.	56.6[h] 67.8[h]	1.08 0.640	$C_{13}H_{26}O_3NCl$	55.80	9.37	5.01	55.87 55.85	9.33 9.40	5.12 4.99

a) Recrystallization solvent: Et=ethyl acetate and ether; EP=ethyl acetate and petroleum ether; A=alcohol and ether.
b) All melting points are uncorrected.
c) Melting point and decomposition points are not sharp.
d) All samples were measured in abs. ethanol at 25—26°C.
All samples exhibited negative rotations except L-phenylgly M·HCl.
e) Prepared from *O*-acetyl DL-serine.
f) Prepared from *O*-benzyl L-serine.
g) Prepared from *O*-benzyl DL-serine.
h) Measured at 17°C.

Experimental

L-α-Phenylglycine *N*-Carboxy Anhydride.—Phosgene was bubbled through a stirred suspension of finely pulverized L-α-phenylglycine[8] (1.51 g. (0.01 mol.) ($[\alpha]_D^{20}$ +156.4°, 1 N HCl)) in 50 ml. of dry dioxane in a water bath at 50°C until a clear solution was obtained. The reaction mixture was then treated with a stream of dry nitrogen for one hour at room temperature in order to expel the unreacted phosgene. The solvent was distilled off in vacuo at 40°C. The residual oily product was crystallized by treating it with petroleum ether. The crystals were filtered, washed with petroleum ether, and dried. The product was recrystallized from ethylacetate and petroleum ether. A yield of 1.45 g. (82%) of L-phenylglycine NCA was obtained; m. p. 114°C. Titration: calcd. for $C_9H_7O_3N$, neut. equiv. 177.2. Found: neut. equiv. 177.5. The neutral equivalent values were determined by titration in methanol with N/50 sodium methoxide, using thymol blue as an indicator.

The NCAs of D-α-phenylglycine and DL-α-phenylglycine were prepared in the same way as has been described above. The specific rotation of the original D-α-phenylglycine[8] was $[\alpha]_D^{20}$ −155.4° (*c* 0.49, 1 N HCl). The yields, melting points, and neutral equivalents of D- and DL-α-phenylglycine NCA[9] are as follows:

	D	DL
Yield, %	84	85
M. p., °C	112	100
Neut. equiv.	175.8	176.5

L-α-Phenylglycine Menthyl Ester Hydrochloride.—To a solution of 1.42 g. (0.008 mol.) of L-α-phenylglycine NCA in 40 ml. of dry dioxane, 6.0 g. of *l*-menthol and 25 ml. of dioxane previously saturated with hydrogen chloride were added at 0°C. After the reaction mixture had been stirred magnetically for 24 hr. at room temperature, the solvent was distilled off in vacuo át 50°C. The residual crystals were treated with petroleum ether, filtered, and dried. The crystals thus obtained were mixed with an 8% sodium hydrogen carbonate solution, and the resulting oil was extracted with ether to obtain the free menthyl ester. The ethereal layer was washed once with water and dried with anhydrous sodium sulfate. To this dried solution, dry hydrogen chloride gas was introduced in order to precipitate the L-α-phenylglycine M·HCl, which was then kept in a refrigerator overnight. The ester hydrochloride crystals were filtered and washed with petroleum ether. L-α-Phenylglycine M·HCl (2.0 g., 77%) was obtained. This was recrystallized from abs. ethanol and ether; m. p. 253—254°C $[\alpha]_D^{25}$ +9.6° (*c* 0.98, ethanol). The analytical data are shown in Table I.

Menthyl ester hydrochlorides of D- and DL-α-phenylglycine were prepared in the way which has been described earlier. The physical and analytical data are shown in Table I.

O-Benzyl L-Serine *N*-Carboxy Anhydride.[10]—*O*-Benzyl L-ser NCA was synthesized by the phosgene method in the same way as α-phenylglycine NCA. Yield 81%, m. p. 71—72°C.

Found: neut. equiv., 222.5; N, 6.43. Calcd. for $C_{11}H_{11}O_4N$: neut. equiv., 221.2; N, 6.33%.

***O*-Benzyl-L-serine Menthyl Ester Hydrochloride.**—A mixture of *O*-benzyl-L-ser NCA[10] (4.4 g. (0.02 mol.) in 10 ml. of dry dioxane) and *l*-menthol (10.0 g. in 15 ml. of dry dioxane) was mixed with 80 ml. of ether which had been saturated with hydrogen chloride at 0°C. This mixture was then stirred at room temperature for four days. After the evaporation of the solvent under reduced pressure, the residual syrup was dissolved in 200 ml. of petroleum ether, and the resulting *O*-benzyl-L-ser M·HCl was extracted with water three times. The aqueous layers were combined, and sodium hydrogen carbonate was added in order to precipitate the oily free ester. The free ester was extracted with ether twice, and the ether solution was dried with anhydrous sodium sulfate and filtered. To the filtrate, dry hydrogen chloride gas was introduced, and the ether was evaporated. After the crystallization of *O*-benzyl-L-ser M·HCl, petroleum ether was added. The crystals were filtered and dried. *O*-Benzyl L-ser M·HCl (5.0 g.) was obtained (68%); m. p. 125—130°C. This was recrystallized from ethyl acetate and petroleum ether; m. p. 132—133°C, $[\alpha]_D^{17}$ −47.5° (*c* 1.11, ethanol). The analytical data are shown in Table I.

***O*-Benzyl-DL-serine Menthyl Ester Hydrochloride.**—A mixture of *O*-benzyl-DL-ser NCA[7] (5.1 g. (0.023 mol.) in 10 ml. of dioxane) and menthol (12.0 g. in 15 ml. of dioxane) was mixed with 80 ml. of ether which had been saturated with hydrogen chloride at 0°C. The reaction mixture was treated in the same way as that of the L-serine derivative.

O-Benzyl-DL-ser M·HCl (5.8 g.) was obtained (68%); m. p. 135—151°C, $[\alpha]_D^{17}$ −46.5° (*c* 1.01, ethanol). This was recrystallized from ethylacetate and ether. Three fractions were isolated by the recrystallization procedure: fraction I, 2.0 g., m. p. 181—182°C, $[\alpha]_D^{17}$ −46.0° (*c* 1.13, ethanol), fraction II, 1.80 g., $[\alpha]_D^{17}$ −47.0° (*c* 0.962, ethanol) m. p. 135—150°C, and fraction III, 1.50 g., $[\alpha]_D^{17}$ −46.8° (*c* 0.960, ethanol), m. p. 128—130°C. No melting point depression was observed in the mixed melting point test of fraction III with *O*-benzyl L-ser M·HCl. Elemental analyses of carbon, hydrogen, and nitrogen of fractions II and III also agreed with the theoretical values.

L-Serine Menthyl Ester Hydrochloride.—The hydrogenolysis of *O*-benzyl-L-ser M·HCl (1.0 g. in 100 ml. of 80% ethanol) was carried out with 2.0 g. of 5% palladium on charcoal for 12 hr. After the reaction had been completed, the catalyst was removed by filtration. The filtrate was then evaporated in order to crystallize the ester hydrochloride. The crystals were dissolved in water containing excess sodium hydrogen carbonate. The liberated free ester was extracted with ether. The ether solution was washed once with water, dried with anhydrous sodium sulfate, and filtered. Dry hydrogen chloride gas was introduced into the ether

8) E. Fischer and O. Weichhold, *Ber.*, **41**, 1292 (1908).
9) H. Leuchs and W. Geiger, ibid., **41**, 1721 (1908).
10) Zvi Bohak and E. Katchalski *Biochemistry*, **2**, 228 (1963).

solution in order to crystallize L-serine M·HCl. The crystals were filtered, washed with ether, and dried. L-Serine M·HCl (0.50 g.) was obtained (66%); m. p. 216—219°C (decomp.), $[\alpha]_D^{17}$ −69.8° (*c* 0.702, ethanol). The analytical data are shown in Table I.

DL-Serine Menthyl Ester Hydrochloride I.—*O*-Benzyl-DL-serine M·HCl (fraction I; m. p. 181—182°C, 0.5 g.) was dissolved in 50 ml. of 80% ethanol. In order to remove the benzyl group, hydrogenolysis was carried out with 1.0 g. of 5% palladium on charcoal for 12 hr. The reaction product was treated in the same way as that of the L-serine derivative; 0.34 g. (90%) of DL-serine M·HCl was thus obtained, m. p. 164—166°C, $[\alpha]_D^{17}$ −56.6° (*c* 1.08, ethanol). The analytical data are shown in Table I.

DL-Serine Menthyl Ester Hydrochloride II.—*O*-Benzyl-DL-ser M·HCl (fraction III: m. p. 128—130°C, 0.50 g.) was treated in the manner which has been described above. When debenzylated serine M·HCl was recrystallized from ethanol and ether, 0.20 g. of the product was obtained; m. p. 217—219°C (decomp.), $[\alpha]_D^{17}$ −67.8° (*c* 0.640, ethanol). The mixed melting point test with L-ser M·HCl did not show any melting point depression. The analytical data are shown in Table I.

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