[CONTRIBUTION FROM THE NATIONAL CANCER INSTITUTE, THE NATIONAL INSTITUTES OF HEALTH[1]]

SOME NEW N-ACYL DERIVATIVES OF ALANINE AND PHENYLALANINE

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Received July 23, 1952

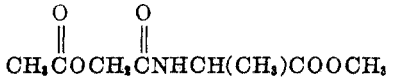
In connection with studies underway in this laboratory on the splitting of various N-acylated amino acids by the enzyme Acylase I (1), prepared from hog kidneys, a number of new N-acyl-DL-alanines and -DL-phenylalanines have been prepared and characterized. These are listed in Table I together with data on their melting points and analyses.

All of the substituted N-benzoylalanines were prepared by a Schotten-Baumann reaction between the corresponding acid chlorides and the appropriate amino acid in the presence of excess alkali. The N-dichloroacetyl- and the N-trichloroacetyl-alanine were obtained by slight modifications of this procedure.

The fluoroacetyl derivatives (X and XIII) were obtained by reaction of ethyl fluoroacetate with the proper amino acid ester to yield the ethyl ester of the fluoroacetyl amino acid. The esters on treatment with one equivalent of alkali followed by acidification yielded the N-fluoroacetyl amino acids.

Reaction of the sodium salt of methanethiol with the ethyl ester of the appropriate N-chloroacetyl amino acid followed by treatment of the resulting ester with one mole of alkali led to the N-(methylmercaptoacetyl) amino acids (XI and XIV).

The barium salt of N-hydroxyacetyl-DL-alanine (XII) was prepared from N-bromoacetyl-DL-alanine by conversion of the latter to its methyl ester which was in turn treated with silver acetate to give what was presumably the O-acetyl derivative of N-hydroxyacetyl-DL-alanine methyl ester (XVIII).



XVIII

This intermediate was not characterized, though a similar derivative in the phenylalanine series was, but it was treated with two moles of alkali to yield the salt of the desired hydroxyacetyl-DL-alanine. Similar treatment of the intermediate isolated in the phenylalanine series did not lead to an analytically pure compound.

The N-trifluoroacetyl amino acids were obtained by reaction of trifluoroacetic anhydride with the corresponding amino acid according to the method of Weygand[2] and Csendes (2).

Also characterized in this work were a number of new compounds used in the

[1] Public Health Service, Federal Security Agency.

[2] The author is indebted to Dr. Weygand for making the details of this procedure available prior to their publication.

TABLE I

N-Acyl Amino Acids

No.	Compound	Method of prep.	Yield[b], %	M.P.,[c] °C.	Empirical formula	Analyses[d]							
						Calculated				Found			
						C	H	N	X[e]	C	H	N	X[e]
I	*p*-Chlorobenzoyl-DL-alanine	A	35	180–182	$C_{10}H_{10}ClNO_3$	52.8	4.4	6.2	15.6	52.8	4.5	6.2	15.7
II	*p*-Fluorobenzoyl-DL-alanine	A	40	160–162	$C_{10}H_{10}FNO_3$	56.8	4.7	6.6		57.0	4.9	6.7	
III	*p*-Bromobenzoyl-DL-alanine	A	55	185–187	$C_{10}H_{10}BrNO_3$	44.1	3.7	5.1	29.4	44.2	4.0	5.3	29.5
IV	*p*-Iodobenzoyl-DL-alanine	A	60	198–200	$C_{10}H_{10}INO_3$	37.6	3.1	4.4		37.7	3.4	4.5	
V	*p*-Anisoyl-DL-alanine	A	22	178–180	$C_{11}H_{13}NO_4$	59.2	5.8	6.3		59.7	6.1	6.2	
VI	*m*-Anisoyl-DL-alanine	A	75	156–158	$C_{11}H_{13}NO_4$	59.2	5.8	6.3		59.2	6.2	6.3	
VII	2,4-Dichlorobenzoyl-DL-alanine	A	30	137–139	$C_{10}H_9Cl_2NO_3$	45.8	3.4	5.3	27.1	45.7	3.4	5.4	26.9
VIII	Dichloroacetyl-DL-alanine	A_1	45	169–171	$C_5H_7Cl_2NO_3$	30.0	3.5	7.0	35.5	30.2	3.9	7.0	35.3
IX	Trichloroacetyl-DL-alanine	A_2	13	161.5–162.5	$C_5H_6Cl_3NO_3$	25.6	2.6	6.0	45.3	25.8	2.9	6.1	45.1
X	Fluoroacetyl-DL-alanine	B and C	17	109–111	$C_5H_8FNO_3$	40.2	5.4	9.4		40.5	5.8	9.3	
XI	Methylmercaptoacetyl-DL-alanine	D	68	110.5–112	$C_6H_{11}NO_3S$	40.7	6.2	7.9	18.1	40.8	6.5	7.8	17.8
XII	Hydroxyacetyl-DL-alanine	E	9	—	$(C_5H_8NO_4)_2Ba \cdot H_2O$	26.9	4.0	6.3	30.7	27.2	4.2	6.4	30.6
XIIA	Bromoacetyl-DL-alanine[f]	A_1	15	112–114	$C_5H_8BrNO_3$	28.6	3.8	6.7	38.1	28.2	3.9	6.5	38.4

XIII	Fluoroacetyl-DL-phenylalanine	C	92	144–146	$C_{11}H_{12}FNO_3$	58.7	5.3	6.2		58.7	5.4	6.2		
XIV	Methylmercaptoacetyl-DL-phenylalanine	D	73	109–111	$C_{12}H_{15}NO_3S$	56.9	5.9	5.5	12.6	56.5	6.2	5.6	12.4	
XV	Trifluoroacetyl-DL-phenylalanine	F	51	127–128	$C_{11}H_{10}F_3NO_3$	50.6	3.8	5.4		51.2	4.3	5.4		
XVI	Trifluoroacetyl-DL-proline	F	27	81–83	$C_7H_8F_3NO_3$	39.8	3.8	6.6		40.0	4.1	6.8		
XVII	Trifluoroacetyl-D-alanine[f, g]	F	13	66–68	$C_5H_6F_3NO_3$	32.4	3.2	7.6		32.6	3.5	7.7		

[a] The yields reported are not necessarily optimum. No attempt was made to find the reaction conditions leading to maximum yields. [b] The melting point given was constant for at least two crystallizations from the same solvent. [c] Analyses by R. J. Koegel and staff of the National Cancer Institute. [d] X = Cl, Br, S, or Ba. [e] This compound has been reported previously but was not characterized. Abderhalden and Abderhalden, *Fermentforschung*, **16**, 48 (1938). [f] $[\alpha]_D^{25}$ +60.6°. *c*, 2% in water. The compound was extremely hygroscopic. [g] Trifluoroacetyl-L-alanine, $[\alpha]_D^{25}$ −60.9°; *c*, 2% in water; m.p. 66–68° was also prepared. *Anal.* C, 32.6; H, 3.6; N, 7.7.

syntheses outlined above. Details of their preparation and properties are given in the experimental section.

EXPERIMENTAL[3]

*N-(p-Iodobenzoyl)-*DL*-alanine* (method A). DL-Alanine (9 g.) was dissolved in 120 ml. of 2 *N* sodium hydroxide and 26 g. of *p*-iodobenzoyl chloride [prepared from the acid by the method (3) used for the bromo derivative] was added. The mixture was shaken until a clear solution was obtained and was then placed in the ice-box overnight. The precipitated sodium salt was collected, dissolved in hot water, and precipitated by the addition of hydrochloric acid. The collected precipitate, 25 g., m.p. 193–195°, was recrystallized from water-alcohol and then washed with ether to give 19 g. (60%) of N-*p*-iodobenzoyl)-DL-alanine, m.p. 198–200°.

The remaining substituted benzoyl alanines were obtained in a similar manner except that the sodium salt did not usually precipitate and the N-benzoylamino acid was isolated by acidification of the reaction mixture.

*N-Dichloroacetyl-*DL*-alanine* (method A_1). This procedure differs from method A only in that one equivalent of alkali and the amino acid were placed in the reaction flask, surrounded by ice, and then the remaining alkali and the acid chloride dissolved in ether were added simultaneously and dropwise to the contents of the flask. The product was isolated by extraction with ethyl acetate.

*N-Trichloroacetyl-*DL*-alanine* (method A_2). This procedure is the same as method A_1 except that the ether was omitted.

*N-Fluoroacetyl-*DL*-phenylalanine ethyl ester* (method B). Phenylalanine ethyl ester (18 g.) and 13.5 g. of ethyl fluoroacetate (4) were refluxed 2 hours and the alcohol formed was removed through a short 25-cm. glass helice-packed column. The low-boiling material was removed and the residue was distilled at reduced pressure to give 13 g., b.p. 145–150° at 1 mm. The distillate, which partially crystallized, was taken up in ethyl acetate and washed with dilute hydrochloric acid and sodium bicarbonate solution; the organic layer was dried and the solvent was concentrated. Addition of petroleum ether gave 6.6 g. (28%) of product, m.p. 73–75°.

Anal.[4] Calc'd for $C_{13}H_{16}FNO_3$: C, 61.7; H, 6.3; N, 5.5.
Found: C, 61.7; H, 6.7; N, 5.6.

*N-Fluoroacetyl-*DL*-phenylalanine* (method C). To 5.1 g. of N-fluoroacetyl-DL-phenylalanine ethyl ester in 20 ml. of ethanol there was added 0.8 g. of sodium hydroxide in 20 ml. of water and the mixture was allowed to stand overnight. Most of the alcohol was removed in a stream of air; the residue was acidified and extracted with ethyl acetate. Concentration of the solvent yielded 4.2 g. (92%) of the desired acid, m.p. 143–145°. Recrystallization from ethyl acetate raised the melting point to 144–146°.

The analogous alanine derivative was prepared in the same way except that the intermediate ester was not characterized.

*N-(Methylmercaptoacetyl)-*DL*-alanine* (method D). Methanethiol (4 g.) was passed into a solution of 1.3 g. of sodium in 130 ml. of absolute ethanol. The solution was then heated to reflux and 9.7 g. of N-chloroacetyl-DL-alanine ethyl ester (5) in 50 ml. of ethanol was added dropwise. After stirring for 30 minutes the sodium chloride formed was removed and the filtrate was concentrated to about 50 ml. To this concentrate 2 g. of sodium hydroxide in 20 ml. of water was added and the mixture was allowed to stand overnight. The alcohol was removed and the reaction worked up as in the previous cases to yield 7 g. of crystals, m.p. 109–112°. After recrystallization from ethyl acetate plus petroleum ether (b.p. 35–65°), 6 g. of N-(methylmercaptoacetyl)-DL-alanine, m.p. 110.5–112° was obtained.

The phenylalanine analog was prepared in a similar way from N-chloroacetyl-DL-phenylalanine ethyl ester.

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[3] All melting points are corrected.

[4] Analyses by R. J. Kogel and staff of the National Cancer Institute.

*N-Chloroacetyl-*DL*-phenylalanine ethyl ester.* This compound was prepared in 14% yield from the free acid by direct esterification with ethanol and anhydrous hydrogen chloride, m.p. 71–74°.

Anal.[4] Calc'd for $C_{13}H_{16}ClNO_3$: C, 57.8; H, 5.9; N, 5.2; Cl, 13.2.
Found: C, 56.9; H, 5.9; N, 5.3; Cl, 13.0.

*N-Bromoacetyl-*DL*-alanine methyl ester.* N-Bromoacetyl-DL-alanine (24 g.) was esterified with excess diazomethane (6) in the usual way. There was thus obtained 19 g. (74%) of ester, m.p. 60–63°; upon recrystallization from ethyl acetate and petroleum ether the m.p. was raised to 62–64°.

Anal.[4] Calc'd for $C_6H_{10}BrNO_3$: C, 32.1; H, 4.5; N, 6.2.
Found: C, 32.0; H, 4.5; N, 6.0.

*Hydroxyacetyl-*DL*-alanine, barium salt* (method E). N-Bromoacetyl-DL-alanine methyl ester (5.5 g.) and 5 g. of silver acetate were refluxed 2 hours in 100 ml. of acetic acid following which the solution was filtered and concentrated *in vacuo* to a thick syrup. The syrup was taken up in ethyl acetate, washed with bicarbonate solution, dried, the solvent removed, and the residue distilled to give 3.6 g. of material b.p. 125–130° at 1 mm. This material, which was presumably the acetate of the hydroxyacetylalanine methyl ester (XVIII), in 20 ml. of ethanol was allowed to stand overnight with 1.4 g. of sodium hydroxide in 20 ml. of water. The alcohol and water were removed *in vacuo*; the residue was acidified, extracted with ethyl acetate, and the solvent was then removed. Neutralization of the residue to *p*H 6 with barium hydroxide followed by addition of acetone resulted in crystallization of the barium salt. Recrystallization from water and acetone gave 0.5 g. (9%) of the Ba salt of N-hydroxyacetyl-DL-alanine.

*N-[O-(acetyl)hydroxyacetyl]-*DL*-phenylalanine methyl ester.* N-Bromoacetyl-DL-phenylalanine methyl ester (7) (10 g.) and 6 g. of silver acetate were refluxed 2 hours in 100 ml. of acetic acid. The inorganic salts were removed by filtration; the acetic acid was removed by distillation *in vacuo* and the residue was extracted with ethyl acetate. The extract was washed with sodium bicarbonate, dried, and the solvent was removed. Distillation of the residue gave 8 g. (86%) of the desired ester, b.p. 200–205° at 1 mm.

Anal.[4] Calc'd for $C_{14}H_{17}NO_5$: C, 60.2; H, 6.1; N, 5.0.
Found: C, 60.1; H, 6.2; N, 5.1.

Attempts to convert this ester to the Ba salt of the hydroxyacetyl derivative in a manner analogous to that used for the derivative of alanine did not lead to a pure product.

*N-Trifluoroacetyl-*DL*-phenylalanine* (method F). Following the procedure Weygand and Csendes (2) used for preparing the analogous alanine derivative, 7.5 g. of DL-phenylalanine was reacted with 6.4 ml. (9.5 g.) of trifluoroacetic anhydride to give 6 g. (51%) of product, m.p. 119–123°. Recrystallization from benzene raised the m.p. to 125.5–127°.

The other trifluoroacetyl derivatives were prepared similarly.

SUMMARY

A number of new N-acylated derivatives of DL-alanine and DL-phenylalanine have been prepared and characterized.

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