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THE REACTIVITY OF THE N-ACYLAMINO ACID *p*-NITROPHENYL ESTERS

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Abstract—The ionization constants of eleven benzyloxycarbonylamino acids and rate constants of aminolysis and base-catalysed hydrolysis of their *p*-nitrophenyl esters were measured in dioxane-water (1:4). The correlation of σ^* of side chain group R and reactivity of amino acids and their derivatives is discussed.

CHEMICAL properties of α-amino acids in aqueous medium are determined by their dipolar structure $\overset{+}{N}H_3CHRCO\overset{-}{O}$[1] and by the chemical nature of the side chain groups (R). Unlike the corresponding carboxylic acids[2] and amines,[3] the ionization constants of both carboxyl and amino groups of α-amino acids with non ionizable side chain groups are not influenced by the nature of R.[1] This phenomenon should be associated with strong electrostatic interaction between the two charged groups in the dipolar structure. Removal of one of the charges may reveal the effect of the chemical nature of side chain group (R). The N-acylation of α-amino acids could be used for this purpose. Unfortunately, the corresponding quantitative kinetic and equilibria data are not available yet, in spite of the great importance of reactions of α-amino acids and their derivatives.

Many reactions of α-amino acid derivatives are of great biological importance (biosynthesis and chemical synthesis of peptide bond, enzymatic hydrolysis of proteins). In general these reactions may be considered as nucleophilic substitutions at the carbon atom of the activated carboxyl group of the α-amino acid derivative;

$$Z—NHCHRCO—X + YH \rightarrow Z—NHCHR—CO—Y + HX$$

where YH is the nucleophilic reagent, X the activating group.

The main purpose of this work was to study the relative reactivity of α-amino acid derivatives with different R groups. The kinetics of hydrolysis and aminolysis (with glycylglycine as amine component $HY = H_2NCH_2CONHCH_2COO^-$) of benzyloxycarbonylamino acid *p*-nitrophenyl esters ($Z = C_6H_5CH_2OCO—$, $X = —OC_6H_4NO_2$) were studied in alkaline aqueous media. In addition the pK_a values of corresponding N-benzyloxy-carbonylamino acids were measured.

RESULTS AND DISCUSSION

In aqueous solutions of glycylglycine, two parallel reactions of the *p*-nitrophenyl ester take place: (1) aminolysis and (2) base catalysed hydrolysis with formation of

[1] E. J. Cohn and J. T. Edsall, *Proteins, Amino Acids and Dipolar Ions*. Reinhold, N.Y. (1942).

[2] R. W. Taft, in *Steric Effects in Organic Chemistry* (Edited by M. S. Newman,) J. Wiley, New York, Chapman and Hall, London (1956).

[3] H. K. Hall, *J. Amer. Chem. Soc.* **79**, 5441 (1957).

benzyloxycarbonyl derivatives of tripeptide and amino acid respectively, *p*-nitrophenol being formed in both reactions.

(1) $C_6H_5CH_2OCONHCHRCOOC_6H_4NO_2 + NH_2CH_2CONHCH_2COO^- \xrightarrow{k_A}$
$C_6H_5CH_2OCONHCHRCONHCH_2CONHCH_2COO^- + HOC_6H_4NO_2$

(2) $C_6H_5CH_2OCONHCHRCOOC_6H_4NO_2 + OH^- \xrightarrow{k_H}$
$C_6H_5CH_2OCONHCHRCOO^- + HOC_6H_4NO_2$

where k_A and k_H represent the rate constants of aminolysis and hydrolysis respectively. The reaction products and free glycylglycine were identified by paper electrophoresis (pyridine–acetate buffer pH 5·6, 25 v/cm, 2 h). The rate equation of *p*-nitrophenyl ester consumption following (1) and (2) is:

$$-\frac{d[E]}{dt} = k_A[RNH_2][E] + k_H[OH^-][E] = k[E]$$

where $[E]$ is the *p*-nitrophenyl ester concentration and k the first order effective rate constant. As an example the effective rate constants of benzyloxycarbonylglycine *p*-nitrophenyl ester, consumption are plotted against total glycylglycine concentration (C_A) in Fig. 1. These data show the linear proportionality of k to the total amine concentration. Thus the effective rate constant of *p*-nitrophenol formation is expressed by:

$$k = k_A'C_A + k_H'$$

From data shown in Table 1 it is evident that a similar pattern is typical for derivatives of all amino acids investigated. Except the first-order rate constants—Table 1 contains values of k_A' and k_H'—effective amynolysis and hydrolysis rate constants were calculated by the method of least squares.

The hydrolysis rate is known as a sum of the rates of basic, neutral and acidic hydrolyses:

(3) $$k_H' = k_{OH^-}[OH^-] + k_{H_2O} + k_{H_3O^+}[H_3O^+]$$

where k_{OH^-}, k_{H_2O} and $k_{H_3O^+}$ are corresponding rate constants. The measurement of the hydrolysis rate of benzyloxycarbonylglycine *p*-nitrophenyl ester in the range of pH 8·80–pH 10·5 (Fig. 2) has shown that all terms in Eq. (3) except the first one, could be neglected, e.g. $k_H = k_{OH^-}[OH^-]$. The calculated hydroxyl ion concentration at pH 8·80, t 25° in the presence of 20% dioxan is $1{\cdot}52 \cdot 10^{-6}$ M (the salt effect being not taken into consideration).

The aminolysis rate constants were calculated as $k_A = (C_A/[RNH_2])k_A'$ where $(C_A/[RNH_2]) = 1{\cdot}304$ for experimental conditions. The aminolysis and hydrolysis rate constants k_A, k_{OH^-} and the ionization constants of benzyloxycarbonylamino acids are listed in Table 2.

The benzyloxycarbonylamino acids are more acidic than the corresponding carbonic acids. In Fig. 3, values of pK_a of benzyloxycarbonylamino acids are plotted against polar constants σ^* of the side chain groups. The Taft equation is in good accordance with experimental data for ionization constants of benzyloxycarbonyl derivatives of valine, isoleucine, leucine, alanine, phenylalanine, tyrosine, methionine,

TABLE 1. KINETIC DATA FOR AMINOLYSIS OF *p*-NITROPHENYL ESTERS OF BENZYLOXYCARBONYL DERIVATIVES OF DIFFERENT AMINO ACIDS (μ 0·8; t 25°; 20%; DIOXAN v/v)

C_A (M)	$K^{=}_{obs}$ (sec^{-1})	C_A (M)	$K^{=}_{obs}$ (sec^{-1})	C_A (M)	$K^{=}_{obs}$ (sec^{-1})
Glycine		*Isoleucine*		*Proline*	
0·01	0·00178	0·02	0·000118	0·02	0·000207
0·02	0·00269	0·04	0·000174	0·04	0·000399
0·03	0·00404	0·06	0·00253	0·06	0·000556
0·04	0·00491	0·08	0·00365	0·08	0·000788
0·06	0·00750				
0·08	0·00967				
$K^{=}_{A}{}' = 11{\cdot}69 \cdot 10^{-2}$ sec^{-1} M^{-1}		$K^{=}_{A}{}' = 4{\cdot}1 \cdot 10^{-3}$ sec^{-1} M^{-1}		$K^{=}_{A}{}' = 9{\cdot}50 \cdot 10^{-3}$ sec^{-1} M^{-1}	
$K^{=}_{H}{}' = 4{\cdot}39 \cdot 10^{-4}$ sec^{-1}		$K^{=}_{H}{}' = 2{\cdot}25 \cdot 10^{-5}$ sec^{-1}		$K^{=}_{H}{}' = 1{\cdot}25 \cdot 10^{-5}$ sec^{-1}	
Alanine		*Phenylalanine*		*Aspartic acid β-methyl ester*	
0·02	0·00138	0·02	0·00153	0·01	0·00356
0·04	0·00256	0·04	0·00266	0·02	0·00490
0·06	0·00369	0·06	0·00389	0·03	0·00651
0·08	0·00483	0·08	0·00522	0·04	0·00770
$K^{=}_{A}{}' = 5{\cdot}76 \cdot 10^{-2}$ sec^{-1} M^{-1}		$K^{=}_{A}{}' = 6{\cdot}01 \cdot 10^{-2}$ sec^{-1} M^{-1}		$K^{=}_{A}{}' = 14{\cdot}0 \cdot 10^{-2}$ sec^{-1} M^{-1}	
$K^{=}_{H}{}' = 2{\cdot}34 \cdot 10^{-4}$ sec^{-1}		$K^{=}_{H}{}' = 2{\cdot}52 \cdot 10^{-4}$ sec^{-1}		$K^{=}_{H}{}' = 2{\cdot}16 \cdot 10^{-3}$ sec^{-1}	
Valine		*Tyrosine*		*O-acetylserine*	
0·02	0·000135	0·02	0·00153	0·01	0·00372
0·04	0·000226	0·04	0·00278	0·02	0·00720
0·06	0·000336	0·06	0·00365	0·03	0·00935
0·08	0·000415	0·08	0·00514	0·04	0·01150
$K^{=}_{A}{}' = 4{\cdot}75 \cdot 10^{-2}$ sec^{-1} M^{-1}		$K^{=}_{A}{}' = 5{\cdot}87 \cdot 10^{-2}$ sec^{-1} M^{-1}		$K^{=}_{A}{}' = 2{\cdot}55 \cdot 10^{-1}$ sec^{-1} M^{-1}	
$K^{=}_{H}{}' = 4{\cdot}05 \cdot 10^{-5}$ sec^{-1}		$K^{=}_{H}{}' = 3{\cdot}40 \cdot 10^{-4}$ sec^{-1}		$K^{=}_{H}{}' = 1{\cdot}57 \cdot 10^{-3}$ sec^{-1}	
Leucine		*Methionine*			
0·02	0·00101	0·01	0·00101		
0·04	0·00181	0·02	0·00156		
0·06	0·00256	0·04	0·00271		
0·08	0·00358	0·06	0·00377		
		0·08	0·00537		
$K^{=}_{A}{}' = 4{\cdot}22 \cdot 10^{-2}$ sec^{-1} M^{-1}		$K^{=}_{A}{}' = 5{\cdot}92 \cdot 10^{-2}$ sec^{-1} M^{-1}			
$K^{=}_{H}{}' = 1{\cdot}35 \cdot 10^{-4}$ sec^{-1}		$K^{=}_{H}{}' = 3{\cdot}44 \cdot 10^{-4}$ sec^{-1}			

glycine and aspartic acid methyl esters. The σ^* values were taken from the literature.[2] The polar constant of the $—CH_2CH_2SCH_3$ group, the side chain of methionine was calculated using $\sigma_1 = 0{\cdot}2$ for $—SCH_3$ group[4] $\sigma^*_{-CH_2CH_2SCH_3} = 1/2{\cdot}8^2\ 6{\cdot}2\ \sigma^* = 0{\cdot}15$. The polar constant of $—CH_2COOCH_3$ group (aspartic acid β-methyl ester side chain) is equal $\sigma^*_{-CH_2COOCH_3} = 1/2{\cdot}8\ \sigma^*_{-COOCH_3} = 0{\cdot}79$. The reaction constant $\rho^* = 1{\cdot}05$ was calculated by multiplying the slope of the Taft plot by factor 2·8 which accounts for the deterioration of the inductive effect of side chain group (R) when transmitting it through the α-carbon atom.

It is necessary to note that our ρ^* value is lower than $\rho^* = 1{\cdot}72$ for carboxylic acids.[2] The correlation between ionization constants and inductive effect of the side chain group is indicative for constancy of the benzyloxycarbonylamino group inductive effect in this reaction series, and for absence (or constancy) of interaction between

[4] M. Charton, *J. Org. Chem.* **29**, 1222 (1964).

TABLE 2. pK_a OF BENZYLOXYCARBONYLAMINO ACIDS $C_6H_5CH_2OCONHCHRCOOH$ AND THE RATE CONSTANTS OF AMINOLYSIS ($K_A^=$) AND HYDROLYSIS ($K_{OH^-}^=$) OF *p*-NITROPHENYL ESTERS OF BENZYLOXYCARBONYLAMINO ACIDS $C_6H_5CH_2OCONHCHRCOOC_6H_4NO_2$

	Derivative of amino acid	R—	σ^*	pK_a	$K_A^= \cdot 10^2$ sec^{-1} . M^{-1}	$K_{OH^-}^= \cdot 10^{-2}$ sec^{-1} . M^{-1}
1	Glycine	—H	+0·49	4·15	14·82*	2·88
2	Alanine	—CH_3	0	4·34	7·43	1·54
3	Valine	—$CH(CH_3)_2$	0·19	4·48	0·61	0·26
4	Leucine	—$CH_2CH(CH_3)_2$	−0·225	4·42	5·44	0·89
5	Isoleucine	—$CH(CH_3)(C_2H_5)$	−0·125	4·45	0·53	0·15
6	Phenylalanine	—$CH_2C_6H_5$	+0·215	4·25	7·75	1·66
7	Tyrosine	—$CH_2C_6H_4OH$	+0·110	4·30	7·57	2·23
8	Methionine	—$CH_2CH_2SCH_3$	+0·15	4·28	7·64	2·26
9	Aspartic acid β-methyl ester	—CH_2COOCH_3	+0·79	4·05	18·1	14·2
10	O-Acetylserine	—CH_2OCOCH_3	+1·26	3·88	32·9	10·3
11	Proline	—	—	4·28	1·23	0·08

* This value is the average of $K_A^= = 14{\cdot}68 \cdot 10^{-2}$ sec^{-1}, $K_A^= = 15{\cdot}08 \cdot 10^{-2}$ sec^{-1} M^{-1} and $K_A^= = 15{\cdot}08 \cdot 10^{-2}$ sec^{-1} M^{-1} measured at pH 8·28, pH 8·80 and pH 9·10 respectively.

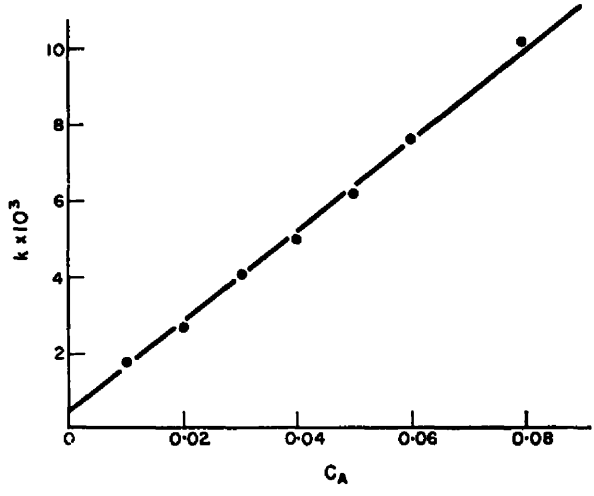

FIG. 1. The rate constants of *p*-nitrophenol formation from benzyloxycarbonylglycine *p*-nitrophenyl ester in glycylglycine buffer (pH 8·80, μ 0·8 M, 20% dioxan v/v,25°) as a function of total amine concentration.

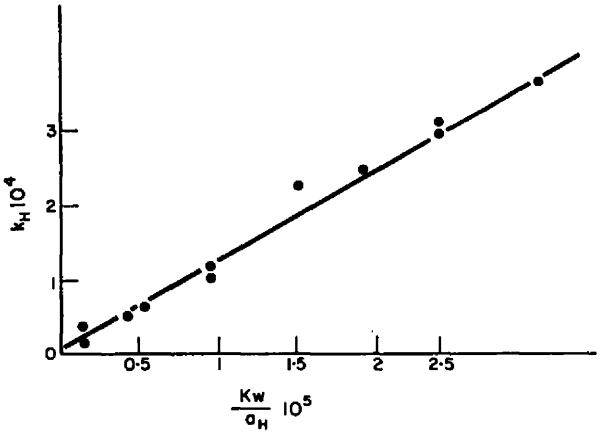

FIG. 2. The hydrolysis rate constants of benzyloxycarbonylglycine *p*-nitrophenyl ester as measured in pH-state (μ 0·8 M, 20% dioxan v/v, 25°).

benzyloxycarbonylamino group and side chain group. The inductive effect of benzyloxycarbonylamino group can be evaluated by comparation of the ionization constants of acetic acid and of benzyloxycarbonylglycine (μ 0·8, t 25°, 20% dioxan v/v) which are equal pK_a 4·15 and pK_a 5·25 respectively.

The substitution of the benzyloxycarbonylamino group for an hydrogen atom in acetic acid brings about a change of the ionization constant by more than ten times

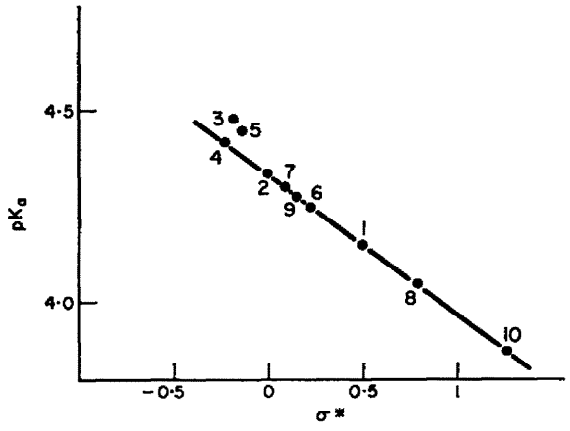

FIG. 3. The Taft correlation of aminolysis rate constants of benzyloxycarbonyl derivatives of amino acids. 1. Glycine; 2. Alanine; 3. Valine; 4. Leucine; 5. Isoleucine; 6. Phenylalanine; 7. Tyrosine; 8. β-Methylaspartate; 9. Methionine; 10. O-Acetylserine.

($\Delta pK_a = 1{\cdot}1$), while substitution of an α-hydrogen atom in benzyloxycarbonylglycine changes the pK_a in the range of $\Delta pK_a = 0{\cdot}6$. Hence the benzyloxycarbonylamino group is more electronegative than any of the side chain groups. The deviations from correlation line in Fig. 3 for valine and isoleucine derivatives related to the steric effects of isopropyl and sec-butyl side chain groups, are not of great importance. The deviations from correlation line do not exceed 0·1 pK units.

N-Acylation of α-amino acids leads to the appearance of the inductive effect of the side chain group on the carboxyl group reactivity in amino acid derivatives. The strong electrostatic interaction in the dipolar molecule is evidently responsible for the absence of the side chain inductive effect on the protolytic equilibria. The following reasons for the absence of the correlation between σ^* of side chain group and ionization constants of free amino acids should be taken into consideration. 1. Direct electrostatic interaction of two charged groups (field effect) and its influence on solvation of the dipolar ion is operative in the ionization reaction. 2. The absence of sensitivity to the inductive effect of the side chain group ($\rho^* \approx 0$) is evident for forward and reverse reactions (protonization and deprotonization of carboxyl group) in the presence of a second very strong electronegative substituent. The influence of inductive and field effects of a very strong electronegative $-NH_3^+$ group (σ^* ca. 7) on the mechanism of transmittance of inductive effect of a side chain group (R) was followed by non additivity of inductive effect (the saturation effect[5]).

[5] R. P. Smith, H. Eyring, *J. Amer. Chem. Soc.* **75**, 5183 (1953).

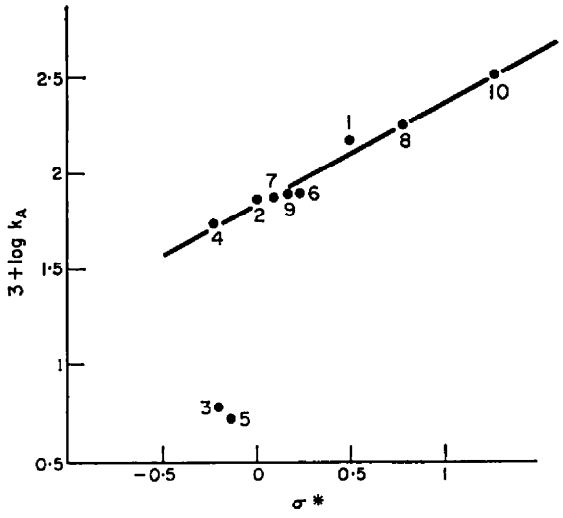

FIG. 4. The Taft correlation of aminolysis rate constants of *p*-nitorphenyl esters of benzyloxycarbonylamino acids. The designation of amino acids see Fig. 3.

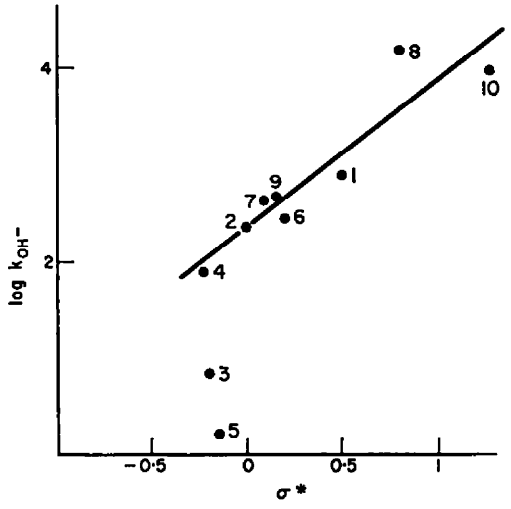

FIG. 5. The Taft correlation of rate constants of hydroxyl-catalysed hydrolysis of benzyloxycarbonylamino acid *p*-nitrophenyl esters. The amino acids designated as in Fig. 3.

The kinetic data in Table 2 show correlation between the reactivity of benzyloxycarbonylamino acid *p*-nitrophenyl esters and σ^* of the side chain group (R). From Figs. 4 and 5 it is evident that $\log k_A$ and $\log k_{OH^-}$ are proportional to the σ^* for all amino acids studied except valine and isoleucine. There is no literature data concerning values of side chains of tyrosine and of O-acetylserine. The approximate σ^* values were interpolated using the pK_a of corresponding benzyloxycarbonylamino acids (Fig. 3). Figures 4 and 5 show that experimental kinetic data agree with the

Taft equation. This suggests that the values of σ^* used are close to real polar constants. The reaction constants of aminolysis is $\rho^* = 1{\cdot}48$ and that of hydrolysis—$\rho^* = 2{\cdot}20$. It should be noted that ρ^* of hydrolysis of *p*-nitrophenyl esters of benzyloxycarbonylamino acids is less than $\rho^* = 2{\cdot}8$ for the hydrolysis of carboxylic acid ethyl esters.[2] The correlation of pK_a of N-acylamino acids and reactivity of their derivatives does not hold only for valine, isoleucine and proline derivatives. The low reactivity of the proline derivative is probably related to the specific structure of this heterocyclic "imino" acid. The retardation of hydrolysis and aminolysis of valine and isoleucine derivatives is related to the steric effect of side chain groups. But in this case the deviations from correlation lines in Figs. 4 and 5 are rather large and equal about one logarithmic unit (0·95 and 0·60 for valine derivatives, 1·05 and 1·0 for isoleucine derivatives correspondingly), as compared with 0·1 unit for ionization reaction. Such an increase in sensitivity to steric effects is probably connected with the bulky *p*-nitrophenyl group rather than with steric properties of a nucleophylic reagent. This is apparent from approximately equal steric effects in reactions of hindered amino acid derivatives with a small particle (hydroxyl ion in hydrolysis) and with a rather bulky molecule (glycylglycine in aminolysis).

EXPERIMENTAL

The benzyloxycarbonylamino acids were prepared by coupling amino acids with benzyloxycarbonyl chloride.[6] Benzyloxycarbonyl derivatives of the following amino acids were synthesized: glycine, m.p. 120° (lit.[6] 120°), D,L-alanine, m.p. 114° (lit.[6] 115°), D,L-valine, m.p. 77° (lit.[7] 76–78°), D,L-leucine, m.p. 53° (lit.[7] 46–49°), D,L-isoleucine m.p. 48°. (Found: C 63·38 H 7·12 N 5·69; $C_{14}H_{19}NO_4$, requires: C 63·38 H 7·22 N 5·28%), D,L-phenylalanine m.p. 103° (lit.[8] 103°), D,L-methionine m.p. 113–114° (lit.[8] 112°), L-proline, m.p. 76–77° (lit.[9] 76–77°). Benzyloxycarbonyl-L-tyrosine, m.p. 96–97° (lit.[10] 94–95°) was prepared by saponification of benzyloxycarbonyl-L-tyrosine ethyl ester. N-benzyloxycarbonyl-β-methyl-D,L-aspartate m.p. 111° (lit.[11] 112°) was prepared by method formerly used for synthesis of N-benzyloxycarbonyl-γ-methyl-L-glutamate.[12] N-benzyloxycarbonyl-O-acetyl-D,L-serine, m.p. 119–120° (lit.[13] 116–118°) was prepared from O-acetyl-D,L,serine and benzyloxycarbonyl chloride. The standard procedures were used for the synthesis of *p*-nitrophenyl esters of benzyloxycarbonyl derivatives of the following amino acids: glycine, m.p. 128–129° (lit.[14] 128°), D,L-alanine m.p. 99° (lit.[15] 101–102°), D,L-valine, m.p. 97° (lit.[15] 98–99°), D,L-leucine, m.p. 76–77° (lit.[15] 76–77°), D,L-phenylalanine, m.p. 104–105°. (Found: C, 65·81; H, 4·98; N, 6·69; $C_{23}H_{20}O_6N_2$ requires: C, 65·71; H, 4·79; N, 6·66%), L-tyrosine, m.p. 156° (lit.[16] 155–157°), L-proline, m.p. 95° (lit.[17] 93–94°). The *p*-nitrophenyl ester of N-benzyloxycarbonyl-β-methyl-D,L-aspartate, m.p. 110–111°, (Found: C, 56·76; H, 4·61; N, 6·82; $C_{19}H_{18}O_8N_2$ requires: C, 56·67; H, 4·51%; N, 6·96%) was prepared by "mixed anhydride" procedure. The *p*-nitrophenyl ester of benzyloxycarbonyl-D,L-isoleucine m.p. 78–79° was prepared. (Found: C, 61·95; H, 5·63; N, 7·24; $C_{26}H_{22}O_6N_2$ requires: C, 62·16; H, 5·74; N, 7·25%.) The *p*-nitrophenyl ester of N-benzyloxycarbonyl-O-acetyl-D,L-serine, m.p. 116°. (Found: C, 56·93; H, 4·50; N, 6·89; $C_{19}H_{18}N_2O_8$,

[6] M. Bergmann and L. Zervas, *Ber. Dtsch. Chem. bes.* **65**, 1192 (1932).
[7] S. W. Fox, M. Fling, H. Wax and C. W. Petting, *J. Amer. Chem. Soc.* **72**, 1862 (1950).
[8] C. A. Dekker and J. S. Fruton, *J. Biol. Chem.* **173**, 471 (1948).
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[17] M. Goodman and K. C. Stueben, *J. Amer. Chem. Soc.* **81**, 3980 (1959).

requires: C, 56·71; H, 4·47; N, 6·96%) was prepared following the procedure proposed for L-isomer.[18] The *p*-nitrophenyl ester of benzyloxycarbonyl-D,L-methionine, m.p. 98–99°, (Found: C, 56·24; H, 4·91; S, 7·94; N, 6·89; $C_{19}H_{20}O_6N_2S$ requires: C, 56·48; H, 4.90; S, 7·92; N, 6·92%) was synthesized according to Wieland.[15]

The ionization constants were determined potentiometrically using an autotitrator TTTl-C (Radiometer) with glass electrode G-202C. All titrations were performed in aqueous solutions with ionic strength μ 0·8, containing 20% dioxan (v/v) in a thermostated cell of the autotitrator at 25° ± 0·1°. The uncorrected pK_a values were always accepted as the pH of half-neutralization. The chart speed was 0·2 pH/cm from which the half-neutralization point was determined with the accuracy of 0·02 pK units.

The hydrolysis rates of benzyloxycarbonylglycine *p*-nitrophenyl ester were measured in the interval of pH 8·8–10·5 by the pH-state method. In each run 2·6 mg of *p*-nitrophenyl ester was dissolved in 20 cc dioxan–water mixture (20% v/v, μ 0·8) which was initially brought to a fixed pH in the thermostated cell of the autotitrator (25°). This fixed pH was kept constant with 0·02 N KOH (in 20% dioxan v/v, μ 0·8). The pseudo-first order rate constants determined from consumption curves of KOH solution are plotted against hydroxyl ion concentration in Fig. 2.

The aminolysis reactions of benzyloxycarbonylamino acid *p*-nitrophenyl esters were carried out in glycylglycine, buffer pH 8·80 in the presence of 20% dioxan (v/v) and μ 0·8 with different concentrations of amine. The water–dioxan solutions were used because of the poor solubility of benzyloxycarbonylamino compounds in water. The constant ionic strength was supported by addition of KCl. The concentration of both charged groups of dipolar form of glycylglycine were taken into account, when ionic strength was calculated. The concentration of the reactive non-dipolar form of glycylglycine was calculated from the following equation:

$$[RNH_2] = C_A \frac{K_A}{K_A + [H_3{}^+O]}$$

where $RNH_2 = NH_2CH_2CONHCH_2COO^-$, C_A is the total concentration of glycylglycine, and K_A the ionization constant of NH_3^+-group of glycylglycine, which is equal pK_a 8·26 in experimental conditions ($[RNH_2] = 0{\cdot}776\ C_A$).

The concentration of hydroxyl ions was calculated from values of water–dioxan ionic products K_w without salt effect correction. The concentration of *p*-nitrophenyl esters was $4 \cdot 10^{-5}$ M in all runs. The total glycylglycine concentrations varied from 0·01 M to 0·08 M. The concentration of hydroxyl ions and reactive glycylglycine form remained constant owing to a large excess of glycylglycine, therefore all runs were first order reactions. The course of the reaction was controlled by the spectrophotometric measurement of *p*-nitrophenol concentration (λ 405 mμ). All measurements were performed in the thermostated at 25° ± 0·1° rectangular fused silica cells, d = 1 cm. Prior to beginning the run, 4 cc of buffer solution with a fixed glycylglycine concentration (μ 1·0) was introduced in the glass-stoppered test tube which was then equilibrated at 24° ± 0·1°; afterwards, 1 cc dioxan solution of *p*-nitrophenyl ester previously equilibrated was added at the same temp. After careful mixing the solution was transferred to the spectrophotometer cell, which was hermetically closed with a polythene sheet and special cover. The optical density measurements were started 20–25 sec after the addition of dioxan solution. The mixing of water and dioxan resulted in a temp rise of the mixture. Therefore both reaction components were previously equilibrated at 24° and not at 25°. The measurements were carried out until at least 90% of *p*-nitrophenyl ester conversion was effected. After the completion of the reaction (at least 8–10 half-periods) the final optical density (d_∞) was measured and constancy of pH of reaction mixture was controlled potentiometrically or spectrophotometrically. Only fully completed runs were considered. The calculated first order rate constants are summarized in Table 1.

[18] M. A. Ondetti, *J. Med. Chem.* **6**, 12 (1963).