749. *Reaction of N-Carboxy-α-Amino-acid Anhydrides with Hydrochlorides of Hydroxylamine, O-Alkylhydroxylamines, and Amines; Syntheses of Amino-hydroxamic Acids, Amido-oxy-peptides, and α-Amino-acid Amides.*

By Yehuda Knobler, Shmuel Bittner, and Max Frankel.

The reaction of anhydrides of *N*-carboxy-α-amino-acids with hydrochlorides of amines, resulting in hydrochlorides of α-amino-acid amides and derivatives, competes successfully with polymerisation of the amino-acid or with solvent interaction. The order of reactivity is the reverse of the basicity of the attacking amine. The dependence of the reaction on solvents, on temperature, on amount of acid present, and on the steric requirements of the amine, has been followed.

Procedures were worked out for syntheses of: (*a*) amido-oxy-peptides of amino-oxyacetic acid, of canaline and its dioxopiperazine, (*b*) amino-hydroxamic acids, and (*c*) *N*-aryl- and *N*-alkyl-amides of amino-acids.

In view of the interest in peptide-like derivatives of amino-oxyacetic acid, we caused phenylalanine *N*-carboxyanhydride to react with ethyl amino-oxyacetate or with its hydrochloric acid salt. Whereas with the free amino-oxy-acid ester the reaction resulted in initiation of a chain polymerisation leading to polymeric polypeptides, the interaction with its salt afforded the desired ethyl phenylalanylamino-oxyacetate.[1] These results induced us to study the factors repressing polymerisation during interaction of anhydrides of *N*-carboxy-α-amino-acids with amines. Salt formation with amino-acid amine, apparently essential for protection against polymerisation, is encountered in the following known conversions of *N*-carboxyanhydride: into amino-acid salt in aqueous hydrochloric acid [2] or in a buffered acidic solution; [3] into amino-acid ester hydrochloride in ethanolic hydrogen chloride; [4] into amino-acid chloride hydrochloride in inert solvents saturated with hydrogen chloride,[5] and into picrates of some amino-acid amides in the presence of amine and picric acid.[6]

Attractive possibilities may arise from the knowledge of the factors determining the course of such reactions, particularly regarding acylation of amine salts. With this in mind, we studied the effects of basicity strength and of steric requirements of the attacking amine, effects of solvent and of temperature, as well as of the amount of acid present.

Reactions of *N*-carboxyanhydrides of α-amino-acids with hydrochlorides of: ethyl amino-oxyacetate, canaline ester and derivatives, hydroxylamine, and aliphatic or aromatic amines, led to smooth syntheses of hydrochlorides of amido-oxy-peptides, of aminohydroxamic acids, and of amino-acid amides. In this reaction, the *N*-carboxyanhydride liberates attacking free amine by forming a new salt of the α-amino-acid derivative involving transfer of hydrogen chloride. This salt formation stops the reaction after the first acylation step.
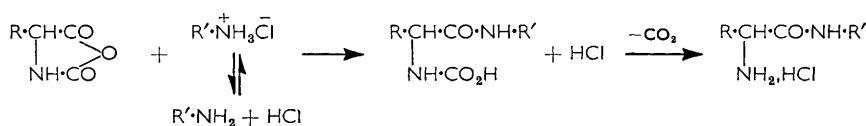
[1] Knobler, Boni, Bittner, and Frankel, *Bull. Res. Counc. Israel*, 1961, **10A**, No. 1, 43.
[2] Wessely, *Z. physiol. Chem.*, 1925, **146**, 72.
[3] Bartlett and Jones, *J. Amer. Chem. Soc.*, 1957, **79**, 2153.
[4] Bergmann, Zervas, and Ross, *J. Biol. Chem.*, 1935, **111**, 245; Katchalski and Spitnik, *J. Amer. Chem. Soc.*, 1951, **73**, 2946; Moore, Dice, Nicolaides, Westland, and Wittle, *ibid.*, 1954, **76**, 2884.
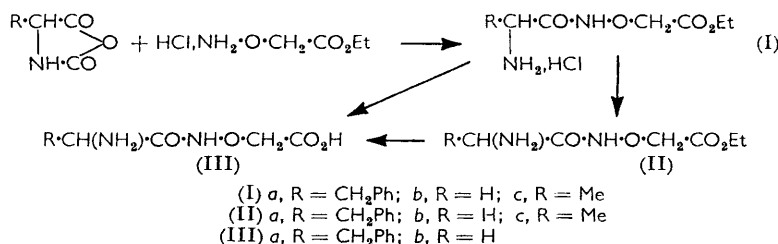[5] Kopple and Katz, *J. Amer. Chem. Soc.*, 1956, **78**, 6199; Brenner and Photaki, *Helv. Chim. Acta*, 1956, **39**, 1525.
[6] Wessely and John, *Monatsh.*, 1927, **48**, 1.

The procedure is technically simple, applicable to a wide range of bases, and yields some amino-acid derivatives unobtainable by reaction of *N*-carboxyanhydride with free bases.

Amido-oxy-peptides of Amino-oxyacetic Acid.—*N*-Carboxyanhydrides were treated with one equivalent of ethyl amino-oxyacetate hydrochloride in 0·1—0·3M solutions at room temperature. Chloroform, dimethylformamide, and ethanol–water (3 : 1) were the most suitable solvents; the reaction occurred also in dioxan, tetrahydrofuran, ethyl acetate, acetic acid, and carbon tetrachloride. Polypeptides resulting from the competitive polymerisation, or the remaining anhydride, were removed, after evaporation of the solvent under a vacuum, by precipitation with water. The amido-oxy-peptide ester hydrochlorides (I) precipitated from the reaction solution or were subsequently isolated after evaporation of the solvent. They were characterised by chromatographic values, by Jaffe's colour test [7] after hydrolysis, and by preparation of benzamido- and tritylamino-derivatives. Phenylalanyl-(I*a*), glycyl-(I*b*), and alanyl-amino-oxyacetic acid ester hydrochloride (I*c*) were prepared.

The free amido-oxy-peptide esters (II) were liberated from their hydrochlorides by triethylamine. Alkaline hydrolysis led to amido-oxy-peptides of amino-oxyacetic acid (III).

$$R{\cdot}CH{\cdot}CO{>}O + HCl,NH_2{\cdot}O{\cdot}CH_2{\cdot}CO_2Et \longrightarrow R{\cdot}CH{\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CO_2Et \quad (I)$$

$$R{\cdot}CH(NH_2){\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CO_2H \longleftarrow R{\cdot}CH(NH_2){\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CO_2Et$$
$$\text{(III)} \qquad\qquad\qquad \text{(II)}$$

(I) *a*, R = CH_2Ph; *b*, R = H; *c*, R = Me
(II) *a*, R = CH_2Ph; *b*, R = H; *c*, R = Me
(III) *a*, R = CH_2Ph; *b*, R = H

Elevation of temperature (60—100°) facilitates dissolution of the salts in solvents like dioxan, tetrahydrofuran, or ethyl acetate, and accelerates both amide formation and polymerisation. Increased dissociation of the conjugate acid ($H_3N^+{\cdot}O{\cdot}CH_2{\cdot}CO_2Et$) increases

TABLE 1.

Effect of solvent and temperature on the interaction of phenylalanine
N-carboxyanhydride with ethyl amino-oxyacetate hydrochloride.

| Solvent	Temp.	Time (hr.)	Yield [a] (%)	M. p.	Solvent	Temp.	Time (hr.)	Yield [a] (%)	M. p.
Chloroform	25°	4	39 [b]	121°	Ethanol–Water 3 : 1	25°	1·5	60 [d]	120°
,,	25	18	56 [b]	122	,,	25	72	58 [d]	119
,,	25	72	74 [c]	123	Acetic acid	25	4	43 [d]	119
,,	35	6	52 [b]	122	,,	25	72	42 [d]	120
,,	60	6	52 [c]	119	Dioxan	25	72	19 [b]	119
Dimethylformamide	0	336	80 [b]	120	,,	100	6	41 [c]	120
,,	25	2	42 [b]	120	Tetrahydrofuran	64	6	52 [c]	120
,,	25	4	59 [b]	120	Ethyl acetate	77 [e]	6	34 [c]	120
,,	25	18	64 [b]	121	Carbon tetrachloride	59 [f]	6	32 [c]	119
,,	25	72	71 [b]	119					
,,	100	6	12 [c]	118					

[a] Expressed in percentage of ethyl phenylalanyl-amino-oxy-acetate (II*a*); yield of the hygroscopic hydrochloride (I*a*) is about 10—12% higher. [b] Reaction incomplete; unreacted phenylalanine *N*-carboxyanhydride was recovered. [c] Reaction completed; in addition to (II*a*) only polymeric material was isolated. [d] Reaction completed; in addition to (II*a*) only phenylalanine was isolated. [e] Reactants insoluble at 25—35°. [f] The reaction proceeded under partial dissolution.

the concentration of the attacking base ($NH_2{\cdot}O{\cdot}CH_2{\cdot}CO_2Et$), but the greater dissociation of the α-ammonium-peptidic conjugate acid ($H_3N^+{\cdot}CH(R){\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CO_2Et$), diverts the reaction from the desired direction. Amido-oxy-peptide ester formation plays

[7] Frankel, Zvilichovsky, and Knobler, preceding Paper.

mainly the role of an initiation reaction, and chain growth proceeds involving the more basic α-amino-group. The overall picture of the reaction approaches that with free bases; reaction of the hydrochloride of ethyl amino-oxyacetate (pK_b 10·7) [7] with phenylalanine *N*-carboxyanhydride shows a relatively slow initiation and a high rate of propagation, leading to polypeptides (see Table I).

Lowering of the temperature changes the ratio between the concentrations of free attacking amine and of α-amino-acid amide, in favour of the weakly basic reactant. The α-amino-group of the amide (pK_b 6·0—6·5), forming the more stable conjugate ammonium acid, becomes more effectively screened. This leads to the preponderance of the reaction involving the weaker base. At 25°, when kept for 3 days in dimethylformamide, 75—80% yield of amido-oxy-peptide ester hydrochloride was obtained, and in chloroform 80—85%. Further decrease in temperature (0°) slows the reaction, affording somewhat better yields after a prolonged time, practically with exclusion of polymerisation. In ethanol–water (or in acetic acid) the reaction is fast at 25°, being complete after 1—2 hours; yields are lower (50—65%) owing to enhanced competitive interaction of the *N*-carboxyanhydride with the solvent.
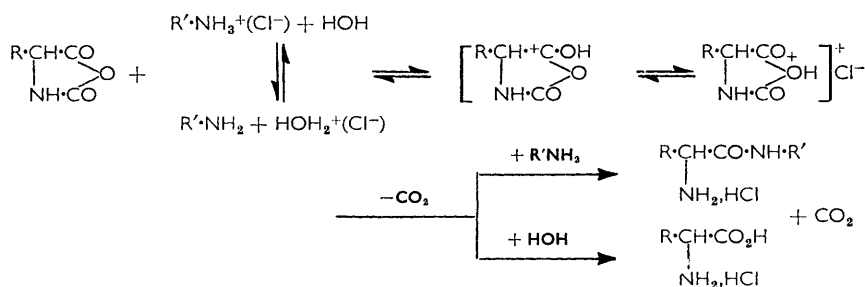
The activation of the anhydride in highly protophilic solvents (water, alcohol, acetic acid) was proved by the reaction of phenylalanine *N*-carboxyanhydride with salts of ethyl amino-oxyacetate or of aniline in the presence of additional amounts of hydrochloric acid. Increase in hydrochloric acid concentration markedly accelerated the reaction, decreased the yields of the amido-oxy-peptide or the amino-acid anilide, and enhanced phenylalanine formation. These results are attributed to the decrease in concentration of the free amine, and to the increased hydrogen-ion concentration. The latter activates the *N*-carboxyanhydrides, which react more quickly with the solvent.

On addition of an excess of the reactant free amine, and at the thus elevated pH, a faster reaction took place giving a higher yield of the amido-oxy-peptide. One equivalent of hydrochloric acid was sufficient to block competitive polymerisation.

As seen above, the reaction occurs much more slowly in polar aprotic and in inert solvents (dimethylformamide, ethyl acetate, tetrahydrofuran, chloroform, dioxan, carbon tetrachloride), than in the amphiprotic solvents. This can be attributed to decreased solvolysis of the amine salt, limiting the amount of free amine as well as the activation of the anhydride by hydrogen ions. Anhydride activation seems also to be reduced by inability to form hydrogen bonds in these solvents. However, the rate-controlling factor appears to be the reduced concentration of the free amine. This can be concluded from experiments using phenylalanine *N*-carboxyanhydride and amine salts in dimethylformamide in the presence of increasing amounts of hydrogen chloride. An appreciable slowing was observed by estimating the unchanged anhydride. Nevertheless, favourable conditions for the desired reaction to take place in these solvents, even if slowly, are provided by: (i) the more efficient blockade of the stronger basic α-amino-group, (ii) the displacement of the weaker base from its hydrochloride by the stronger one, and (iii) the exclusion of solvent interaction.

Yields can also be enlarged by using an excess of the reactant amine salt.

Amido-oxy-peptides of Canaline; Selective Coupling.—Preferred interaction of an *N*-carboxyanhydride with a weaker amine in the presence of a stronger one, in salt form, served for selective coupling with the weaker of two amino-groups contained in the same molecule, *i.e.*, with the amino-oxy-group of canaline. Whereas selective deacylation affords α-*N*-monosubstituted canaline,[8] the interaction of *N*-carboxyanhydride with canaline methyl ester dihydrochloride leads to γ-*NO*-substituted canaline, *e.g.*, *NO*-phenylalanyl-canaline methyl ester dihydrochloride (IV).
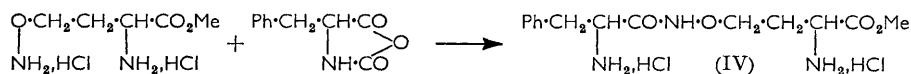
Phenylalanine *N*-carboxyanhydride was also made to react with ethyl α-*N*-benzoyl-canalinate hydrochloride giving ethyl *NO*-phenylalanyl-α-*N*-benzoylcanalinate hydrochloride.

It was reported that 2,5-di(amino-oxymethyl)-3,6-dioxopiperazine and some of its condensation products with oxo-compounds possess biological activity.[9] Our attention was therefore directed to the preparation of homologous compounds, employing methyl canalinate dihydrochloride. The latter was converted into 2,5-di-(2-amino-oxyethyl)-3,6-dioxopiperazine (V*a*) by heating with potassium acetate in ethanol. Treatment of canaline dioxopiperazine (V*a*) with benzaldehyde yielded its biscondensate. Reaction of canaline dioxopiperazine dihydrochloride (V*b*) with two mols. of phenylalanine *N*-carboxy-anhydride yielded the tetrapeptidic 2,5-di-(2-phenylalanylamino-oxyethyl)-3,6-dioxo-piperazine dihydrochloride (V*c*). This tetrapeptidic dioxopiperazine (V*c*) was also pre-pared by dimerisation of ethyl *NO*-phenylalanylcanalinate dihydrochloride (IV).
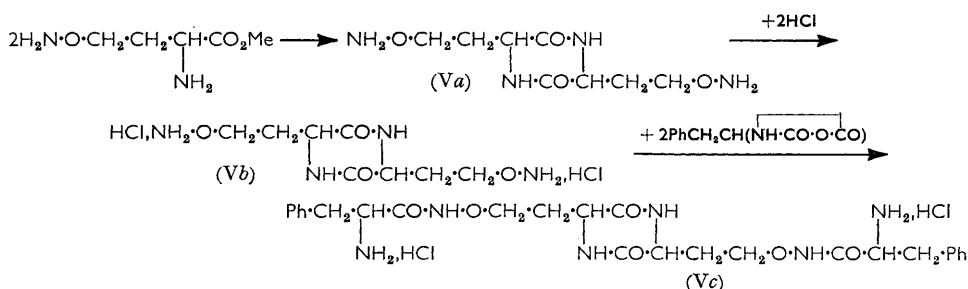
Preparation of Aminohydroxamic Acids.—α-Aminohydroxamic acid hydrochlorides (VI) were prepared from *N*-carboxyanhydrides of amino-acids and hydroxylamine hydro-chloride. The acid hydrochlorides (VI) were converted into the free aminohydroxamic acids (VII). The acids (VII) were also synthesised directly from free hydroxylamine, as in the preparation of β-chloro-α-aminopropiohydroxamic acid.[10]

(VI), (VII) *a*, R = CH$_2$Ph; *b*, R = H; *c*, R = Me; *d*, R = Ph·CH$_2$·O$_2$C·CH$_2$·CH$_2$
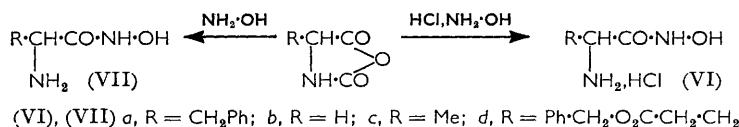
It is noteworthy that, in contrast to the reaction with ethyl amino-oxyacetate, com-petitive polymerisation occurred with hydroxylamine only slightly, even without the blocking effect of the salt formation with hydrogen chloride. This has to be attributed to the increased reactivity of the unhindered hydroxylamine and to the internal salt
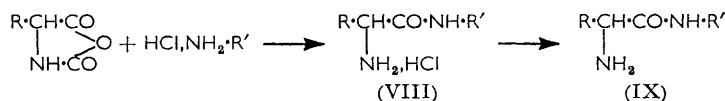
[8] Frankel, Knobler, and Zvilichovsky, *J.*, 1963, 3127.
[9] Michalsky, Ctvrtnik, Horakova, and Bydzovsky, *Experientia*, 1962, **18**, 217.
[10] Plattner, Boller, Frick, Furst, Hegedus, Kirchensteiner, Majnoni, Schlapfer, and Spiegelberg, *Helv. Chim. Acta*, 1957, **40**, No. 160, 1531.

character of the aminohydroxamic acid formed. Hydroxamic acid hydrochlorides of phenylalanine, glycine, alanine, and γ-benzyl-L-glutamic acid (VI*a, b, c, d*) and the corresponding free hydroxamic acids (VII*a, b, c, d*) were prepared. The test with ferric chloride indicated hydroxamic acid formation from amino-acid N-carboxyanhydride and hydroxylamine hydrochloride even in the presence of a five-fold excess of hydrochloric acid in ethanol–water (3 : 1); the same was observed with succinic and acetic acid anhydride. The colour intensity became weaker with increasing hydrochloric acid concentration.

Amino-acid Amides; Basicity and Steric Factors.—The side reactions were found to be dominant with ethylamine hydrochloride despite its reactivity as a free base. Amide formation almost did not occur in water–ethanol, and in dimethylformamide only 21% yield of phenylalanine ethylamide (IX*a*) was obtained. Here, in contradistinction to salts of weak amines, the weaker basicity of the α-amino-group produced leads preferentially to propagation, since ethylamine ($pK_b = 3\cdot3$) forms the stable salt. With other amine hydrochlorides, the yields of amino-acid amides (IX) increased with decreasing basic strength of the reactant amine.

$$\underset{\substack{|\\ \text{NH·CO}}}{\text{R·CH·CO}} \!\!>\!\!O + \text{HCl,NH}_2\text{·R}' \longrightarrow \underset{\substack{|\\ \text{NH}_2\text{,HCl}}}{\text{R·CH·CO·NH·R}'} \longrightarrow \underset{\substack{|\\ \text{NH}_2}}{\text{R·CH·CO·NH·R}'}$$
$$\text{(VIII)}\qquad\qquad\qquad\text{(IX)}$$

R = Ph·CH$_2$; (VIII), (IX) *a*, R′ = Et; *b*, R′ = CH$_2$Ph; *c*, R′ = Ph; *d*, R′ = OMe; *e*, R′ = *p*-C$_6$H$_4$·OMe; *f*, R′ = *o*-C$_6$H$_4$·OMe; *g*, R′ = *p*-C$_6$H$_4$Cl.

Phenylalanine N-carboxyanhydride with benzylamine hydrochloride in dimethylformamide yielded 25—30% of the phenylalanyl-N-benzylamide hydrochloride (VIII*b*) (25% of the free amide IX*b*). With aniline hydrochloride (base strength decrease from pK_b 4·7 to 9·4) 50% of phenylalanylanilide hydrochloride (VIII*c*) (45% of IX*c*) was obtained, despite the known poor aminolytic power of aniline.[11] It should be emphasised that the reaction of phenylalanine N-carboxyanhydride with free aniline leads only to phenylalanyl-phenylanylanilide and to higher polymers.[12]

A clear case of unreactivity of a free amine with an N-carboxyanhydride, only initiating polymerisation of the latter, is the reaction of *p*-chloroaniline (pK_b 12·0) with phenylalanine N-carboxyanhydride. Polymers were precipitated from ethanol–water solution as well as from dimethylformamide, and most of the aromatic amine was recovered. On the other hand use of *p*-chloroaniline hydrochloride leads to high yields of *p*-chloroanilide hydrochloride. This result indicates the influence of a marked difference in the basicity of the end product and of the attacking amine. The weaker the salt forming reactant, the higher the yield of the amidic compound.

Steric inhibition appears to affect profoundly the course of the reaction, as seen with amines of similar basicity. *O*-Methylhydroxylamine hydrochloride, with pK_b equivalent to that of aniline, yielded with phenylalanine N-carboxyanhydride 70% of phenylalanine methoxy-amide hydrochloride (*O*-methyl-N-phenylalanylhydroxylamine hydrochloride) (VIII*d*). With *p*-anisidine hydrochloride the yield of the substituted amide (IX*e*) equals that of the anilide (pK_b 9·0; 9·4); but with the salt of the sterically hindered *o*-anisidine (pK_b 9·6), steric interference decreases the yield to 26%, in spite of the decreased base strength of the amine. A marked increase in yield appears with the salt of the still less basic *p*-chloroaniline (pK_b 12·0), giving 70—75% of phenylalanine *p*-chloroanilide hydrochloride (VIII*g*). The high yields of the amido-oxy-peptide hydrochlorides (I), as described above, are in accordance with the weakness of ethyl amino-oxyacetate as base,[7] and with its small steric hindrance.

No consideration has been paid to specific steric requirements of the N-carboxyanhydrides, or to particulars of solvation.

[11] Smith and Adkins, *J. Amer. Chem. Soc.*, 1938, **60**, 657; Kopple, *ibid.*, 1957, **79**, 662.
[12] Sigmund and Wessely, *Z. physiol. Chem.*, 1926, **157**, 91.

EXPERIMENTAL

Melting points were determined in a Fisher–Johns apparatus.

Infrared spectra were obtained with a Perkin-Elmer Infracord Spectrometer model 137 in Nujol mulls between rock-salt plates.

Chromatography with K.S.G. thin layer (0·25 mm.) on glass plates was employed in the following solvents: (*A*) 80% phenol; (*B*) butan-1-ol–water–acetic acid (4 : 1 : 1). Ninhydrin was the detecting reagent.

pH was measured on a " Radiometer-Copenhagen " titrator with glass and calomel electrodes.

Materials.—Ethyl amino-oxyacetate hydrochloride was prepared as described earlier.[7] *N*-Carboxyanhydrides of phenylalanine and glycine were prepared according to Farthing.[13] Alanine *N*-carboxyanhydride was prepared by the method of Ben-Ishai and Katchalski.[14] γ-Benzyl-L-glutamic acid *N*-carboxyanhydride was prepared by the procedure of Blout and Karlson.[15] Ethyl α-benzamido-γ-amino-oxybutyrate was prepared according to Frankel, Knobler, and Zvilichovsky.[8]

Ethyl Phenylalanylamino-oxyacetate Hydrochloride (I*a*).—Ethyl amino-oxyacetate hydrochloride (1·55 g., 0·01 mole) and phenylalanine *N*-carboxyanhydride (1·91 g., 0·01 mole) were dissolved under anhydrous conditions in chloroform (60 ml.), and the solution was left at room temperature (25°) for 3 days. Impurities were filtered off and the solvent was evaporated under vacuum. The oily residue was shaken with water (30 ml.) and some undissolved polymer was filtered off. The aqueous filtrate was concentrated under a vacuum and the residue was dissolved in ethanol. The hygroscopic *hydrochloride* (2·5 g., 83%) was precipitated by addition of dry ether and cooling to 0°. The product remained hygroscopic even after several recrystallisations. It gave a wine-red colour with ninhydrin (Found: C, 51·2; H, 6·3; N, 9·2; OEt, 14·8; Cl, 11·8. $C_{13}H_{19}N_2O_4Cl$ requires C, 51·6; H, 6·3; N, 9·25; OEt, 14·9; Cl, 11·7%).

Use of an excess of ethyl amino-oxyacetate hydrochloride (2·33 g., 0·015 mole) increased the yield of (I*a*) to 95—97%.

Use of an excess of phenylalanine *N*-carboxyanhydride (2·86 g., 0·015 mole) lowered the yield of (I*a*) to 42%.

Ethyl Glycylamino-oxyacetate Hydrochloride (I*b*).—Ethyl amino-oxyacetate hydrochloride (1·55 g., 0·01 mole) and glycine *N*-carboxyanhydride (1·01 g., 0·01 mole) were dissolved in chloroform (80 ml.) at reflux temperature. After refluxing for 6 hr. and keeping overnight at room temperature, white crystals of the hydrochloride, m. p. 195—196°, were collected. A second crop of the *hydrochloride* (total yield 1·7 g., 80%) was obtained on concentration of the mother-liquor and storage at 0°. Recrystallised from propan-2-ol it melted at 201° (decomp.) [Found: C, 34·1; H, 6·3; N, 13·6; N(Van Slyke), 6·6; OEt, 21·3. $C_6H_{13}N_2O_4Cl$ requires C, 33·9; H, 6·2; N, 13·2; N(Van Slyke), 6·6; OEt, 21·2%].

With dioxan (60 ml.) as solvent, a crude product was obtained; it remained sticky even after several recrystallisations. The yield was 52% (Found: N, 12·8; N(Van Slyke), 6·6; OEt, 22·0%).

Ethyl Alanylamino-oxyacetate Hydrochloride (I*c*).—Alanine *N*-carboxyanhydride (1·15 g., 0·01 mole) and ethyl amino-oxyacetate hydrochloride (1·55 g., 0·01 mole) were refluxed in dioxan (80 ml.) for 6 hr. The mixture was treated as for (I*a*). The *hydrochloride* (1·17 g., 52%) remained hygroscopic even after recrystallisation from ethanol–ether. It gave a red colour with ninhydrin [Found: N, 11·9; N(Van Slyke), 6·1; OEt, 19·9. $C_7H_{15}N_2O_4Cl$ requires N, 12·3; N(Van Slyke), 6·15; OEt, 19·8%].

Ethyl Phenylalanylamino-oxyacetate (II*a*).—Ethyl phenylalanylamino-oxyacetate hydrochloride (3·02 g., 0·01 mole) was dissolved in water (30 ml.), cooled to 5°, and triethylamine was added to pH 8—8·5. The mixture was kept overnight at 0°, while the *amido-oxy-peptide ester* (2·3 g., 86%) separated as fine white crystals, m. p. 119—121°; recrystallised from ethanol, it melted at 123° (Found: C, 58·3; H, 6·8; N, 10·5; OEt, 16·6. $C_{13}H_{18}N_2O_4$ requires C, 58·6; H, 6·8; N, 10·5; OEt, 16·9%); $R_{fA} = 0.85$; $R_{fB} = 0.61$.

Ethyl Glycylamino-oxyacetate (II*b*).—Ethyl glycylamino-oxyacetate hydrochloride (2·1 g., 0·01 mole) was dissolved in water (15 ml.), cooled to 5°, and triethylamine was added dropwise to pH 8—8·5. The aqueous solution was extracted with chloroform (5 × 10 ml.), and the combined organic layer was dried (Na_2SO_4) and concentrated under a vacuum. The *ester*

[13] Farthing, *J.*, 1950, 3213.
[14] Ben-Ishai and Katchalski, *J. Amer. Chem. Soc.*, 1952, **74**, 3688.
[15] Blout and Karlson, *J. Amer. Chem. Soc.*, 1956, **78**, 941.

(1·4 g., 80%) was obtained in white crystals, m. p. 133—134° (decomp.). Recrystallised from ethanol, it melted at 137—138°. It gave a violet colour with ninhydrin (Found: C, 41·2; H, 7·0; N, 15·6; OEt, 25·5. $C_6H_{12}N_2O_4$ requires C, 40·9; H, 6·9; N, 15·9; OEt, 25·5%); $R_{fA} = 0·41$; $R_{fB} = 0·33$.

Ethyl Alanylamino-oxyacetate (IIc).—Ethyl alanylamino-oxyacetate hydrochloride (1·13 g., 0·005 mole) in water (15 ml.) was treated with triethylamine to pH 8—8·5. After extraction with chloroform and concentration under a vacuum, an oily material remained, which crystallised from ethanol. The *ester* (0·57 g., 60%) melted at 155—156°. Its identity with the substance previously prepared[7] was proved by mixed m. p. and infrared spectrum; wine-red colour with ninhydrin.

Ethyl N-*Triphenylmethylphenylalanylamino-oxyacetate*.—To a suspension of ethyl phenylalanylamino-oxyacetate hydrochloride (2·1 g., 0·007 mole) in methylene dichloride (50 ml.), triethylamine (1·62 g., 0·016 mole), and triphenylchloromethane (1·95 g., 0·007 mole) were added. The mixture was stirred vigorously for 30 min., then left overnight at room temperature. The solution was washed twice with water, dried (Na_2SO_4), then concentrated under a vacuum. To the oily residue, a few ml. of absolute ethanol was added, and the solution was concentrated as before. The semi-crystalline *triphenylmethylamido-oxy-peptide ester* (2·5 g., 70%) was recrystallised from methanol, giving crystals of m. p. 144—145° (Found: C, 75·8; H, 6·3; N, 5·7; OEt, 8·5. $C_{32}H_{32}N_2O_4$ requires C, 75·6; H, 6·3; N, 5·5; OEt, 8·8%).

Ethyl N-*Triphenylmethylglycylamino-oxyacetate*.—Prepared as above from ethyl glycylamino-oxyacetate hydrochloride (1·05 g., 0·005 mole), triethylamine (1·2 g., 0·018 mole), and triphenylchloromethane (1·39 g., 0·005 mole), the *product* (1·5 g., 72%) had m. p. 149—150° (Found: C, 72·0; H, 6·4; N, 6·6; OEt, 10·7. $C_{25}H_{26}N_2O_4$ requires C, 71·75; H, 6·3; N, 6·7; OEt, 10·8%).

Ethyl N-*Benzoylphenylalanylamino-oxyacetate*.—Ethyl phenylalanylamino-oxyacetate hydrochloride (1·51 g., 0·005 mole) was dissolved in water (30 ml.) and cooled to 5°. Benzoyl chloride (0·7 g., 0·005 mole) and N-sodium hydroxide (10 ml.) were added dropwise with vigorous stirring during 30 min. Stirring was continued for 1 hr., and the solution was left overnight at 0°. The *benzoyl derivative* (1·25 g., 68%) was filtered off and washed with 0·1N-hydrochloric acid. Recrystallisation from ethyl acetate–light petroleum gave m. p. 143—144° (Found: C, 64·7; H, 6·3; N, 7·85; OEt, 12·5. $C_{20}H_{22}N_2O_5$ requires C, 64·85; H, 6·0; N, 7·6; OEt, 12·2%).

Ethyl N-*Benzoylglycylamino-oxyacetate*.—Prepared as above from ethyl glycylamino-oxyacetate hydrochloride (1·05 g., 0·005 mole), benzoyl chloride (0·7 g., 0·005 mole), and N-sodium hydroxide (10 ml.), the *product* had m. p. 83—85° (Found: C, 55·4; H, 5·5; N, 10·0; OEt, 16·3. $C_{13}H_{16}N_2O_5$ requires C, 55·7; H, 5·75; N, 10·0; OEt, 16·1%).

Phenylalanylamino-oxyacetic Acid (IIIa).—Ethyl phenylalanylamino-oxyacetate (1·33 g., 0·005 mole) was suspended in N-sodium hydroxide (10 ml.) and the mixture left at room temperature for 12 hr. Some impurities were filtered off and the solution was neutralised with N-hydrochloric acid. After addition of ethanol (20 ml.) and keeping overnight at 0°, the *amido-oxy-peptidic acid* (0·8 g., 67%) precipitated. It melted at 186—187° and gave a wine-red colour with ninhydrin [Found: C, 55·2; H, 5·7; N, 11·4; N(Van Slyke), 5·9. $C_{11}H_{14}N_2O_4$ requires C, 55·45; H, 5·9; N, 11·75; N(Van Slyke), 5·75%]; $R_{fA} = 0·18$; $R_{fB} = 0·30$.

Glycylamino-oxyacetic Acid (IIIb).—Prepared as (IIIa), from ethyl glycylamino-oxyacetate hydrochloride (1·05 g., 0·005 mole), the *amido-oxy-peptide* (0·45 g., 61%) had m. p. 155—156°; it gave a violet colour with ninhydrin [Found: N, 18·5; N(Van Slyke), 9·1. $C_4H_8N_2O_4$ requires N, 18·9; N(Van Slyke), 9·45%].

Reaction of Phenylalanine N-*Carboxyanhydride with Ethyl Amino-oxyacetate Hydrochloride.*—*Varying solvent and temperature.* Molar equivalents (0·01 mole) of phenylalanine N-carboxyanhydride and ethyl amino-oxyacetate hydrochloride, were brought to reaction in the solvents listed in Table 1 at 0·25M concentrations. *In the dipolar aprotic solvents and in polyhalogenomethanes*, the reaction was followed by estimation of the yield of the amido-oxy-peptide ester (IIa), and by recovery, if present, of unchanged anhydrides, or by isolation of polymers (see Table 1). Evaporation of the solvent and addition of water to the residue caused precipitation of anhydride, of the polymeric product, or of a mixture of both. The recovered anhydride and the products were identified by m. p., elementary analysis, and infrared spectrum.

The polymeric products gave a positive biuret test, and end group analysis showed them to be oligopeptides, e.g., of an average length of 5—6 units [Found: N(Van Slyke), 1·8; OEt, 5·2.

Ethyl pentaphenylalanylamino-oxyacetate $C_{49}H_{54}N_6O_8$ requires N(Van Slyke), 1·6; OEt, 5·9%] or polypeptides resembling polyphenylalanine [Found: N, 9·5; N(Van Slyke), 0·05; OEt, 0·5. Calc. for $(C_9H_9NO)_n$: N, 9·6%].

In ethanol–water (3 : 1). The reaction rate could be followed by pH measurements and (or) by carbon dioxide evolution. The pH of the solution rose from 1·85 before addition of the anhydride to 3·15 at the end of the reaction. The rise was fast during the first 15 min., slowing down subsequently, and reaching final value after 55—60 min. The rate of carbon dioxide evolution was analogous. The solution was left for a further 15 min., most of the ethanol was evaporated off, water was added, and the solution was neutralised with triethylamine. After filtration from the precipitated amido-oxypeptide ester (II*a*), the filtrate was concentrated; phenylalanine obtained was crystallised from ethanol [Found: N, 8·3; N(Van Slyke), 8·2. Calc. for $C_9H_{11}NO_2$: N, 8·5%]; infrared spectrum identical with that of phenylalanine.

A similar reaction rate (4 hr.) was observed in glacial acetic acid, and phenylalanine was the by-product.

Reaction in the presence of various amounts of hydrochloric acid. (a) Ethyl amino-oxyacetate hydrochloride (1·55 g., 0·01 mole) was dissolved in ethanol–water (3 : 1), and 0·1N-hydrochloric acid was added to pH 1·15 (total volume of the solution 40 ml.). Phenylalanine *N*-carboxy-anhydride (1·91 g., 0·01 mole) was added and pH rise as well as carbon dioxide evolution began after 2—3 min. Final pH 1·5 was reached after 30—35 min., and carbon dioxide evolution ceased.

Addition of 0·5 equiv. of hydrochloric acid before the introduction of the anhydride, accelerated the reaction and gave less ethyl phenylalanylamino-oxyacetate (II*a*) (0·9 g., 33%). Phenylalanine was the by-product. Similar results were obtained with aniline hydrochloride.

(*b*) Decrease of hydrochloric acid concentration, by addition of an excess of ethyl amino-oxyacetate (0·24 g., 0·002 mole) to a 0·25 molar solution of the reactants, raised the pH to 2·55, and completed the reaction in 25 min. (final pH 4·95). The yield of (II*a*) was increased to 72%.

Reaction with Free Ethyl Amino-oxyacetate.—Ethyl amino-oxyacetate was freed from its hydrochloride (1·55 g., 0·01 mole) in ethanol–water (3 : 1) (40 ml.) by triethylamine (1 g., 0·01 mole). Phenylalanine *N*-carboxyanhydride (1·91 g., 0·01 mole) was added at 5°, and the solution was left overnight in the cold. After concentration under a vacuum and addition of water, only a mixture of oligopeptides of a melting range 200—230° (decomp.) were obtained; ninhydrin and biuret reaction positive; Jaffe's test [7] negative [Found: N, 10·4; N(Van Slyke), 2·3; OEt, 8·4. *Ethyl triphenylalanylamino-oxyacetate*, $C_{31}H_{36}N_4O_6$ requires N, 10·0; N(Van Slyke), 2·5; OEt, 8·0%].

Methyl Canalinate Dihydrochloride.—Ethyl α-benzamido-γ-benzamido-oxybutyrate (7·4 g., 0·02 mole) was hydrolysed in 10% hydrochloric acid to give crude DL-canaline dihydrochloride.[16] The oily material was freed from water by repeated addition of portions of acetone and evaporation under a vacuum. The dihydrochloride was dissolved in dry methanol (80 ml.) and the solution was saturated with dry hydrogen chloride and left overnight at room temperature. The solution was concentrated under a vacuum, dissolved again in methanol, and the above procedure was repeated. Crystals remaining from evaporation of methanol were recrystallised from methanol–ether. The *ester dihydrochloride* (3·2 g., 73%) melted at 166—167° (decomp.) (Found: C, 27·3; H, 6·5; N, 12·7; OMe, 14·0. $C_5H_{14}N_2O_3Cl_2$ requires C, 27·2; H, 6·4; N, 12·7; OMe, 14·0%).

Methyl NO-*phenylalanylcanalinate Dihydrochloride* (IV).—Phenylalanine *N*-carboxy-anhydride (0·95 g., 0·005 mole) was added to methyl canalinate dihydrochloride (1·1 g., 0·005 mole) in dry dimethylformamide (70 ml.). The mixture was kept at 40° for 3 days and then concentrated under a vacuum to dryness. Water (50 ml.) was added and most of the material dissolved. Some polymer and unchanged anhydride were filtered off, and water was removed under a vacuum. The crystals of the *γ-amido-oxy-peptide dihydrochloride* (1·0 g., 54%), re-crystallised from methanol–ether, had m. p. 205° (Found: C, 45·9; H, 6·5; N, 11·8; OMe, 8·6; Cl, 18·9. $C_{14}H_{23}N_3O_4Cl_2$ requires C, 45·65; H, 6·3; N, 11·4; OMe, 8·4; Cl, 19·25%).

2,5-Di-(2-amino-oxyethyl)-3,6-dioxopiperazine (V*a*).—Potassium acetate (1·08 g., 0·011 mole) was dissolved in absolute ethanol (20 ml.), methyl canalinate dihydrochloride (1·1 g., 0·005 mole) was added, and the mixture was stirred at 75° until all the solid dissolved. After cooling to room temperature, precipitated potassium chloride was removed and the clear solution was

[16] Knobler and Frankel, *J.*, 1958, 1632.

refluxed for 4 hr., then left overnight at room temperature. The cloudy solution was concentrated, cooled to 0°, and the *dioxopiperazine* (0·4 g., 68%) was collected and washed with small portions of cold ethanol. It had m. p. 116—117° (Found: N, 23·8. $C_8H_{16}N_4O_4$ requires N, 24·1%). The *dihydrochloride* (Vb) was obtained in 85% yield by saturation of an ethanolic solution of the dioxopiperazine with hydrogen chloride and subsequent precipitation with ether (Found: N, 18·3. $C_8H_{18}N_4O_4Cl_2$ requires N, 18·4%). It had $\lambda_{max.}$ (in Nujol) 3·1 μ (NH); 3·7, 4·9 μ (NH_3^+); 6·0 μ (dioxopiperazine CO); second amide band absent.

2,5-*Di*-(2-*benzylideneamino*-*oxyethyl*)-3,6-*dioxopiperazine.*—2,5-Di-(2-amino-oxyethyl)-3,6-dioxopiperazine (1·1 g., 0·005 mole) was dissolved in ethanol (20 ml.) and freshly distilled benzaldehyde (1·4 g., 0·015 mole) was added. The solution was left at room temperature for 30 min., then water was added with cooling and scratching. The *dibenzylidene derivative* (1·8 g., 88%) precipitated and had m. p. 124—125° (Found: C, 64·3; H, 5·6; N, 13·8. $C_{22}H_{24}N_4O_4$ requires C, 64·7; H, 5·9; N, 13·7%). It had $\lambda_{max.}$ (in Nujol) 6·0 μ (second amide band absent).

2,5-*Di*-(2-*phenylalanylamino*-*oxyethyl*)-3,6-*dioxopiperazine Dihydrochloride* (Vc).—(a) To 2,5-di-(2-amino-oxyethyl)-3,6-dioxopiperazine dihydrochloride (Vb) (1·01 g., 0·0025 mole) in ethanol–water (3 : 1) (30 ml.), phenylalanine *N*-carboxyanhydride (0·95 g., 0·005 mole) was added. Evolution of carbon dioxide began immediately and continued until all the anhydride dissolved. The mixture was left overnight at room temperature, concentrated under a vacuum, and to the viscous residue water (20 ml.) was added. Precipitated polymer was filtered off, the solvent was removed under a vacuum, and the residue was washed with small portions of absolute ethanol and with ether. The *tetrapeptide dihydrochloride* (0·9 g., 60%) was still hygroscopic and melted, after recrystallisation from propan-2-ol, at 157—158° [Found: C, 52·0; H, 6·3; N, 13·8; N(Van Slyke), 4·7. $C_{26}H_{36}N_6O_6Cl_2$ requires 52·1; H, 6·1; N, 14·0; N(Van Slyke), 4·7%]; $R_{fA} = 0·45$.

(b) To methyl *NO*-phenylalanylcanalinate dihydrochloride (IV) (1·2 g., 0·003 mole) in ethanol (20 ml.), potassium acetate (0·65 g., 0·006 mole) was added. The mixture was heated at 75°, until all the solid dissolved. Cooling to room temperature (20°) caused precipitation of potassium chloride which was filtered off. The filtrate was refluxed for 4 hr. and left overnight at room temperature; the precipitated *tetrapeptide* (0·5 g., 60%) had m. p. 213—215° (decomp.) [Found: N, 15·6; N(Van Slyke), 5·5. $C_{26}H_{34}N_6O_6$ requires N, 15·9; N(Van Slyke), 5·3%]. It had $\lambda_{max.}$ (in Nujol) 6·05 μ (second amide band absent). The *tetrapeptide dihydrochloride* (Vc) was obtained in 52% yield (based on IV) by saturation of an ethanolic solution of the free tetrapeptide with hydrogen chloride, precipitation with ether, and recrystallisation from propan-2-ol. It melted at 156—157°, and its infrared spectrum was identical to that of (Vc) obtained according to procedure (a).

Ethyl NO-*phenylalanyl*-α-N-*benzoylcanalinate Hydrochloride.*—It was prepared similarly to (IV), from phenylalanine *N*-carboxyanhydride (0·95 g., 0·005 mole) and ethyl α-*N*-benzoyl-canalinate hydrochloride (1·51 g., 0·005 mole). The *amido-oxy-peptide* ester (0·88 g., 39%) remained hygroscopic even after several recrystallisations from ethanol–ether (Found: N, 9·6; OEt, 10·3. $C_{22}H_{28}N_3O_5Cl$ requires N, 9·3; OEt, 10·0%).

Amino-hydroxamic Acid Hydrochlorides (VI). *General Procedure.*—Amino-acid *N*-carboxyanhydride (0·01 mole) was dissolved in ethanol–water (3 : 1) (20 ml.), and hydroxylamine hydrochloride (0·7 g., 0·01 mole) was added. Evolution of carbon dioxide began almost immediately and continued until dissolution of all the solid (20—40 min.). The solution was kept at room temperature for 1 hr. and the solvents were evaporated under a vacuum (40°). The semi-crystalline residue was crystallised from ethanol–ether and, when necessary, recrystallised from propan-2-ol. The hydrochlorides (VI) could be converted into the amino-hydroxamic acids (VII) (described below) by neutralisation with triethylamine in ethanol. The following hydroxamic acid hydrochlorides were prepared: N-(DL-*phenylalanyl*)*hydroxylamine hydrochloride* (VIa) (1·3 g., 60%); m. p. 162—163° (Found: C, 49·7; H, 6·1; N, 12·4; Cl, 16·5. $C_9H_{13}N_2O_2Cl$ requires C, 49·9; H, 6·0; N, 12·9; Cl, 16·4%); $R_{fA} = 0·70$; $R_{fB} = 0·60$; N-*glycylhydroxylamine hydrochloride* (VIb) (0·67 g., 53%); m. p. 107—108° (Found: C, 19·1; H, 5·5; N, 21·7; Cl, 27·65. $C_2H_7N_2O_2Cl$ requires C, 18·9; H, 5·6; N, 22·1; Cl, 28·0%); $R_{fA} = 0·15$; $R_{fB} = 0·25$; N-DL-*alanylhydroxylamine acid hydrochloride* (VIc) (0·6 g.,43 %); m. p. 183—184° [Found: C, 25·3; H, 6·2; N, 19·6; N(Van Slyke), 10·2; Cl, 25·0. $C_3H_9N_2O_2Cl$ requires C, 25·6; H, 6·45; N, 19·9; N(Van Slyke) 9·95; Cl, 25·2%]; $R_{fA} = 0·25$; $R_{fB} = 0·30$; γ-*benzyl*-L-*glutamohydroxamic acid hydrochloride* (VId) (1·85 g., 64%); m. p. 158—159° [Found: C, 49·7; H, 6·0; N, 9·65; N(Van Slyke), 4·5; Cl, 11·8. $C_{12}H_{17}N_2O_4Cl$ requires C, 49·9; H,

5·9; N, 9·7; N(Van Slyke), 4·85; Cl, 12·3%]. $[\alpha]_D^{25} = +34$ ($c = 7\cdot5$ in water); $R_{fA} = 0\cdot75$; $R_{fB} = 0\cdot70$.

Amino-hydroxamic Acids (VII). *General Procedure.*—Amino-acid N-carboxyanhydride (0·01 mole) was dissolved in absolute ethanol (40 ml.) with cooling (5°) and stirring, and a cooled solution of hydroxylamine hydrochloride (0·7 g., 0·01 mole) and triethylamine (1·0 g., 0·01 mole) in ethanol–water (3 : 1) (40 ml.) were added. A strong evolution of gas took place immediately and ceased after a few minutes. Stirring was continued for 2 hr. at 5° and, after keeping overnight at 0°, crystals of the hydroxamic acid precipitated. When necessary, they were recrystallised from water–methanol. The following acids were prepared: N-(DL-phenyl-alanyl)hydroxylamine (VII*a*) (1·5 g., 83%); m. p. 178—180° (decomp.) [17] (Found: C, 59·8; H, 6·7; N, 15·3. Calc. for $C_9H_{12}N_2O_2$: C, 60·0; H, 6·7; N, 15·5%); N-glycylhydroxylamine (VII*b*) (0·7 g., 77%); m. p. 139—140° [17] (Found: C, 26·8; H, 6·5; N, 30·9. Calc. for $C_2H_6N_2O_2$: C, 26·7; H, 6·7; N, 31·1%); N-DL-alanylhydroxylamine (VII*c*) (0·6 g., 57%); m. p. 162—163° [17] (Found: C, 34·4; H, 7·6; N, 26·7. Calc. for $C_3H_8N_2O_2$: C, 34·6; H, 7·7; N, 26·9%); γ-benzyl-L-*glutamohydroxamic acid* (VII*d*) (1·3 g., 52%) m. p. 125—126° (Found: N, 10·9. $C_{12}H_{16}N_2O_4$ requires N, 11·1%).

General Procedure for the Preparation of Amino-acid Amide Hydrochlorides (VIII) *and of Amino-acid Amides* (IX).—Amine hydrochloride (0·01 mole) was added to amino-acid N-carboxyanhydride (0·01 mole) in dry dimethylformamide (15—25 ml.), and the solution was left at room temperature for 3 days. The solvent was evaporated under a vacuum (50—60°), water was added and undissolved material was filtered off. Water was evaporated under a vacuum, and the amino-acid amide hydrochloride was crystallised from ethanol–ether or from chloroform.

The amide was liberated from its hydrochloride by addition of N-sodium hydroxide to the aqueous solution, to pH 8—8·5. The aqueous solution was extracted with ether (4 × 20 ml.) and the combined organic layer was dried (Na_2SO_4), and evaporated to dryness. Oily residues crystallised on keeping overnight at 0°, and, when necessary, the amide was recrystallised from propan-2-ol–water.

In ethanol–water (3 : 1) as solvent, yields were 30—40% lower and phenylalanine was the second product.

The following compounds were prepared: *phenylalanine* N-*methoxyamide hydrochloride* (VIII*d*) (1·6 g., 69%); m. p. 179—180° (Found: C, 52·4; H, 6·3; N, 12·5; $C_{10}H_{15}N_2O_2Cl$ requires C, 52·1; H, 6·55; N, 12·1%); $R_{fB} = 0\cdot55$; *phenylalanine* N-*benzylamide* (IX*b*) (0·6 g., 24%); m. p. 63—64° [Found: C, 75·4; H, 7·1; N, 11·1; N(Van Slyke), 5·6. $C_{16}H_{18}N_2O$ requires C, 75·6; H, 7·1; N, 11·0; N(Van Slyke), 5·5%]; $R_{fA} = 0\cdot90$ $R_{fB} = 0\cdot65$; phenyl-alanine anilide (IX*c*) (1·1 g., 45%); m. p. 78—79° [12] (Found: C, 74·8; H, 6·7; N, 11·7. Calc. for $C_{15}H_{16}N_2O$: C, 75·0; H, 6·7; N, 11·65%); $R_{fA} = 0\cdot90$; $R_{fB} = 0\cdot78$; [At 0°, 80% of the N-carboxyanhydride was recovered, prolongation of reaction time (0°) to four weeks, yielded 28% of the anilide (IX*c*)]; *Phenylalanine* N-*ethylamide* (IX*a*) (0·4 g., 21%); oily product (Found: N, 14·3. $C_{11}H_{16}N_2O$ requires N, 14·6%); $R_{fB} = 0\cdot44$; *Phenylalanine* p-*anisidide* (IX*e*) (1·2 g., 44%); m. p. 89—90° (Found: N, 10·2. $C_{16}H_{18}N_2O_2$ requires N, 10·4%); *Phenyl-alanine* o-*anisidide* (IX*f*) (0·7 g., 26%); m. p. 229—230° (Found: N, 10·1. $C_{16}H_{18}N_2O_2$ requires N, 10·4%); *Phenylalanine* p-*chloroanilide* (IX*g*) (1·8 g., 66%); m. p. 104—105° (Found: C, 65·8; H, 5·6; N, 10·0; Cl, 13·1. $C_{15}H_{15}N_2OCl$ requires C, 65·6; H, 5·5; N, 10·2; Cl, 12·9%); $R_{fB} = 0\cdot75$.

Formation of Phenylalanine Anilide in Presence of an Excess of Hydrogen Chloride.—To hydrogen chloride (0·22 g., 0·006 mole) in dimethylformamide (50 ml.), aniline hydrochloride

TABLE 2.

Recovered anhydride (%) and yields of the anilide (IX*c*) [a] without (*A*) and with (*B*) a 30% excess of hydrogen chloride.

	2 hr.	4 hr.	24 hr.	72 hr.	7 days	10 days
A	66(13) [c]	42(25)	35(35)	(45) [b]	—	—
B	75(5)	66(8)	50(20)	39(32)	32(43)	26(52)

[a] Yields of the anilide are given in parentheses.   [b] Reaction completed; polymer was the second product.   [c] Higher yield than with 1 equiv. of hydrogen chloride; reaction still incomplete.

[17] Cunningham, Newbold, Spring, and Stark, *J.*, 1949, 2091.

(2·6 g., 0·02 mole) was added, followed by phenylalanine *N*-carboxyanhydride (3·82 g., 0·02 mole). After timed intervals, samples of the solution were treated as above; the slowing down of the reaction rate, checked by comparison with the interaction without an excess of hydrogen chloride, at the same molar concentrations, was followed as seen in Table 2. Recovered anhydride and the anilide formed were estimated.

Results with ethyl amino-oxyacetate hydrochloride in presence of excess of hydrogen chloride were similar.

DEPARTMENT OF ORGANIC CHEMISTRY,
THE HEBREW UNIVERSITY OF JERUSALEM, ISRAEL.