phate (1); guanosine 5′-triphosphate (1); uridine 5′-triphosphate (1); and yeast extract (0.5 mg.). Coenzyme A, the triphosphates, and yeast extract were also tested in the presence of added L-alanine and had no significant effect.

Acknowledgment.—The author wishes to thank Professor James Bonner for the opportunity of working in his laboratories, and he wishes to thank Professor Bonner, Mr. John M. Clark, Jr., Dr. Peter H. Lowy and Dr. Richard S. Schweet for many stimulating discussions.

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[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY, UNIVERSITY OF CHICAGO]

The Reaction of Amines with Amino Acid N-Carboxy Anhydrides

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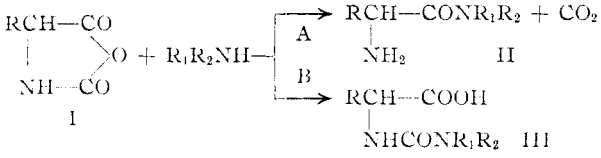
The reaction of amines with N-carboxyanhydrides of α-amino acids proceeds to yield either a ureido acid (attack at the 2-carbonyl of the oxazolidinedione ring) or an amino amide (attack at the 5-carbonyl). The actual course of the reaction is dependent on the steric requirements of the amine, its concentration and its basicity. A number of new ureido acids are reported.

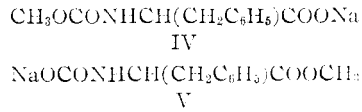
The reaction of an α-amino acid N-carboxy anhydride (4-alkyl-2,5-oxazolidinedione, I, abbreviated here to NCA) with an amine may, in principle, take either of two paths, A or B.



Path A, attack at the 5-carbonyl leading to an α-amino amide (*via* an intermediate carbamic acid derivative), is a normal chain propagation step in the polymerization of I to yield poly-α-amino acids.[1] Path B, although occurring much less frequently under normal circumstances, has been reported as an important chain-terminating reaction in such polymerizations.[2] In view of current interest in the polymers prepared from NCA's, some observations of the path of reaction between the NCA's and various amines are here reported.

The use of reaction path A to provide amides of amino acids is well established. Sigmund and Wessely have reported a 90% yield of phenylalanine amide and a 73% yield of the corresponding ethylamide obtained by treatment of phenylalanine NCA with excess amine in ethyl acetate solution.[3] The dimethylamides of glycine, DL-alanine, DL-phenylalanine and sarcosine have been prepared by action of excess dimethylamine on the corresponding N-carboxyanhydride.[4] The reaction of phenylalanine NCA with two equivalents of aniline yields di- and polypeptide anilides along with the amino acid amide.[5] By reaction in the presence of picric acid, however, the same reactants afford only phenylalanine anilide picrate.[5] The reaction of NCA's with amino acid and dipeptide esters has also been reported, the principal products observed being diketopiperazines and di- and tripeptide esters.[6]

A clear-cut case of reaction *via* path B is found in the reaction of phenylalanine NCA with two equivalents of sodium methoxide. A 3% yield of the urethan IV is obtained in addition to the ester V.[7]



In the present work, N-carboxyanhydrides were treated with a large excess of each of several amines; the ureido acid III formed by reaction along path B was isolated. In most cases the amino amide II, product of path A, was isolated as the picrate. With DL-phenylalanine NCA and aliphatic amines, the ratio of III to II observed was found to be related to the steric requirements of the attacking amine; with *t*-butyl and diethylamine the yield of ureido acid reached preparative values, but with dimethylamine and ethylamine, the amino amide was the dominant product. Glycine NCA behaved similarly, indicating that the benzyl group of the phenylalanine derivative is not an important factor in directing the course of reaction. Sarcosine NCA reacted only by path A, yielding no ureido acid, whatever the amine. The actual results of these experiments are given in Table I.

The facts just cited, taken alone, would indicate that polymerization of phenylalanine NCA could not proceed very far, for the amino group of a growing peptide chain, which must react by path A to continue the chain, cannot have steric requirements less than those of isopropylamine; isopropylamine, according to Table I, reacts by path B about one-half of the time. However, the amino group at the end of a peptide chain during polymerization is neither so strongly basic (pK_a of the amino group of glycine amide, 7.93[8]; that of isopropylamine, 10.72[9]) nor present in so high a concentration as

(1) D. H. G. Ballard and C. H. Bamford, *Proc. Roy. Soc. (London)*, **223A**, 495 (1954).

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(3) F. Sigmund and F. Wessely, *Z. physiol. Chem.*, **157**, 91 (1926).

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(5) F. Wessely and M. John, *Monatsh.*, **48**, 1 (1927).

(6) J. L. Bailey, *J. Chem. Soc.*, 3464 (1950).

(7) A. Berger, M. Sela and E. Katchalski, *J. Org. Chem.*, **25**, 1554 (1953).

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(9) G. Bredig, *Z. physik. Chem.*, **13**, 289 (1894).

TABLE I

YIELD (%) OF UREIDO ACIDS IN THE REACTION OF AMINES WITH N-CARBOXY ANHYDRIDES. EFFECT OF AMINE[a]

Amine	NCA gly	NCA sar	NCA phe
Diethyl	(100)[b]	..	80
t-Butyl	90	0	60
i-Propyl	45	0	35
Ethyl	(55)[b]	..	10 (73)[d,e]
Dimethyl	Low[c]	Low[c]	2.5 (92)[d]
Phenyl	..	..	0 (85)[d]

[a] 0.5 g. of NCA in 10 ml. of 1:1 methylene chloride–amine, 0°. [b] Not isolated in pure, crystalline form. [c] Reference 4. [d] Yield of corresponding amino amide II; isolated as picrate. [e] Reference 3.

TABLE II

EFFECT OF SOLVENT AND CONCENTRATION ON THE REACTION OF AMINES WITH DL-PHENYLALANINE NCA[a]

Amine	Solvent	III, %	Peptide, %
t-Butyl	1:1 amine:CH_2Cl_2	60	0
t-Butyl	Dioxane	9	45[b]
t-Butyl	$(CH_3)_3N$	*ca.* 6	*ca.* 50
Dimethyl	1:1 amine:CH_2Cl_2	0	0
Dimethyl	Dioxane	0	0
Phenyl	1:1 amine:CH_2Cl_2	0	15
Phenyl	1:1 amine:$(CH_3)_3N$	0	15

[a] 0.5 g. of NCA plus 2 equivalents of amine in 10 ml. of solvent. [b] Average chain length 4 residues.

the amino group of isopropylamine under the conditions of the present experiment.

From Table I, it is seen that aniline (pK_a 4.58), a much weaker base than the aliphatic amines, reacts entirely along path A. Some peptide is also formed in this case, indicating that aniline, although present in large excess, is not so reactive toward the NCA as is the more strongly basic amino group of peptide or amino amide. The importance of amine concentration is seen from Table II. Although *t*-butylamine, in large excess, produces 60% of the theoretical amount of ureido acid when acting on phenylalanine NCA, the same amine used in the stoichiometric quantity (two molar equivalents) affords only 9%. In the first case no peptide is formed, but a high yield of peptide is obtained in the second. The unsuccessful competition of *t*-butylamine with peptide amino groups for reaction with the NCA shows, as might be expected, that steric effects, as well as basicity with regard to a proton, must be considered in the reaction with the NCA carbonyls. In dilute solution dimethylamine, having smaller spatial demands, affords no peptide and no ureido acid, only phenylalanine dimethylamide.

The effect of a strongly basic solvent, trimethylamine, was investigated in some instances. Such a solvent is apparently without effect on the course of reaction.

The kinetics of the base-initiated polymerization of NCA's, which proceeds by reaction path A, has been thoroughly discussed by Ballard and Bamford.[1] Their results support the scheme represented on the right side of Figure 1, the rate-determining step for polymerization of phenylalanine NCA being k_A. If the whole of the Figure is admitted as a plausible reaction scheme, enough variables are at hand to explain the results reported in this paper.

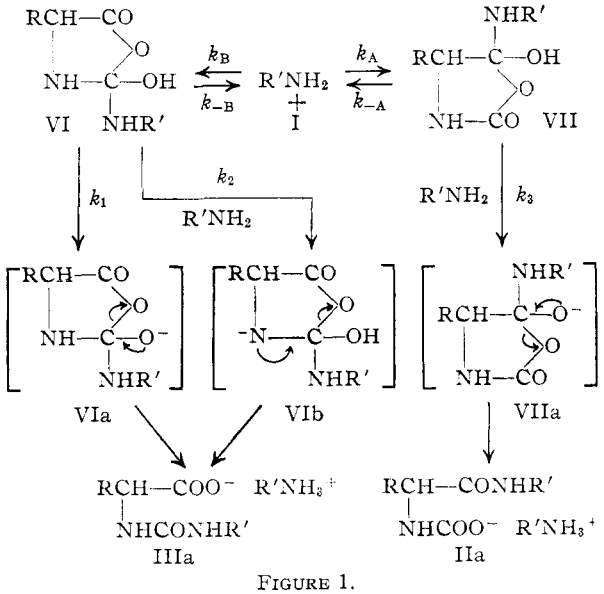

FIGURE 1.

It is necessary to assume that for a relatively weakly nucleophilic amine such as aniline, or for other amines in low concentrations k_A and k_B remain the rate-determining steps. Since the 5-carbonyl is the more highly polarized, k_A is doubtless larger than k_B and a preponderance of reaction by path A is to be expected. With the highly hindered amines, it may be difficult for a second molecule of amine to approach the complexes VI or VII, so that the concentrations of these intermediates build up and the rate of reaction becomes dependent on the removal of a proton from them. The complex VI offers an additional site from which a proton may be removed to complete the reaction, so that its rate of decomposition to products is increased relative to that of VII, thus increasing the amount of NCA which is converted to ureido acid. Sarcosine NCA, having a methyl group in the ring nitrogen, cannot react *via* VIb; further, its 2-carbonyl is probably less polarized than that of phenylalanine NCA so that the concentration of VI may be relatively less. The lack of product arising by path B from sarcosine NCA is thus not surprising.

A number of ureido acids and α-amino amides not previously reported are described in Table III.

Experimental

Materials.—The amines used, except dimethylamine and trimethylamine, were distilled before use. Dimethylamine was used directly from the cylinder in which it was shipped: trimethylamine was distilled from a 25% methanolic solution, dried over potassium hydroxide and redistilled from potassium hydroxide. DL-Phenylalanine-N-carboxyanhydride, m.p. 128–129°, and sarcosine-N-carboxyanhydride, m.p. 104–105°, were prepared by treatment of a dioxane suspension of the amino acid with excess phosgene[10] and were recrystallized from dioxane–petroleum ether and chloroform–petroleum ether, respectively. Glycine-N-carboxyanhydride was prepared by thionyl chloride treatment of carbobenzyloxyglycine[11] and recrystallized from ethyl acetate.

Reaction of Amines with DL-Phenylalanine-N-carboxyanhydride.—To a suspension of 0.50 g. (0.0026 mole) of DL-

(10) D. Coleman, *J. Chem. Soc.*, 3222 (1950).

(11) Y. Go and H. Tani, *Bull. Chem. Soc. Japan*, **14**, 510 (1939); *C. A.*, **34**, 1971 (1940).

TABLE III

A. UREIDO ACIDS (III), $RCH(NHCONR_1R_2)COOH$

R	R^1	R^2	M.p.,[a] °C.	Analyses,[b] % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
H	H	*i*-C_3H_7	166.0–166.5	44.99	45.00	7.55	7.35	17.49	17.09
H	H	*t*-C_4H_9	146.0	48.26	48.24	8.10	8.00	16.08	15.95
$C_6H_5CH_2$	H	*i*-C_3H_7	143.5–144.0	62.38	62.53	7.25	7.12		
$C_6H_5CH_2$	H	*t*-C_4H_9	153.0–154.0	63.61	63.21	7.63	7.62	10.60	10.49
$C_6H_5CH_2$	CH_3	CH_3	133.0–134.0					11.86	11.66
$C_6H_5CH_2$	C_2H_5	C_2H_5	103.5–104.5	63.61	63.60	7.63	7.77	10.60	10.50
CH_3	C_2H_5	C_2H_5	120.0–121.0	51.04	51.17	8.57	8.49	14.89	14.85
i-C_4H_9	C_2H_5	C_2H_5	109.0–109.5	57.36	57.68	9.63	9.87	12.17	12.06

B. PICRATES OF α-AMINO AMIDES (II), $C_6H_2(NO_2)_3OH \cdot H_2NCHRCONR^1R^2$

R	R^1	R^2	M.p.,[a] °C.	Nitrogen,[b] % Calcd.	Found
$C_6H_5CH_2$	C_2H_5	C_2H_5	242–245	15.59	15.30
$C_6H_5CH_2$	H	*t*-C_4H_9	222–224.5	15.59	15.57
$C_6H_5CH_2$	H	*i*-C_3H_7	224–225	16.09	15.80

[a] Given m.p.'s of picrates and ureido acids are decomposition points, except in the case of those ureido acids in which R^1 and R^2 are both alkyl groups. [b] Analyses by Mr. William Saschek of this Laboratory and by Micro-Tech Laboratories, Skokie, Ill.

phenylalanine NCA in 5 ml. of methylene chloride, cooled by an ice-bath, was added 5 ml. (≥0.050 mole) of amine, also at 0°. The NCA dissolved immediately on addition of the amine; some gas evolution occurred. After storage at 0° for 3–4 hr., the solution was allowed to remain at room temperature overnight. In experiments with *t*-butylamine and isopropylamine significant quantities of insoluble material had formed within an hour of mixing.

The reaction mixture was freed of solvent and excess amine by distillation (steam distillation at reduced pressure was used in experiments involving aniline). The residual oil or solid was then distributed between 15 ml. of 2 *N* hydrochloric acid (gas evolution) and three 30-ml. portions of methylene chloride. The organic extracts were dried over magnesium sulfate and concentrated to dryness. After traces of solvent had been removed by storage under vacuum, the crystalline ureido acids were weighed; yields are reported in Table I. Recrystallization of the products so obtained was carried out from chloroform–petroleum ether; analytical data are reported in Table III. The infrared spectra[12] of the ureido acids derived from primary amines were characterized by carboxyl absorption at 1730 $cm.^{-1}$ (usually a doublet) and amide bands at 1625 and 1575 $cm.^{-1}$. (In the reaction of DL-phenylalanine NCA with aniline, an amorphous solid, insoluble in methylene chloride or aqueous acid, appeared during the distribution mentioned above and was collected by filtration. Its infrared spectrum was that of poly-DL-phenylalanine.)

The aqueous acid layer, containing amino acid amide, was cooled and made strongly alkaline with 4 *N* sodium hydroxide before again being extracted with three 50-ml. portions of methylene chloride. The organic phase was dried and concentrated as earlier; the residue was treated with an excess of picric acid as a saturated solution in ethanol. Immediate precipitation of the corresponding picrates occurred. These were dried and weighed and, where necessary, recrystallized from ethanol.

Those experiments involving other than the methylene chloride–amine solvent system were performed by pouring onto the NCA a solution of the amine in dioxane or trimethylamine. Where dioxane was the solvent, the reaction mixtures were held just above the freezing point (10°) for 2 hr. and then allowed to stand at room temperature overnight. With trimethylamine as solvent, the reaction mixture was held at 0° for 4 hr. and the trimethylamine then allowed to evaporate as the solution warmed to room temperature. The isolations of products were carried out as before. However, in the experiments utilizing only two equivalents of *t*-butylamine it was necessary to separate the initial methylene chloride extract (from acid solution) into two fractions. This extract was concentrated to about 30 ml. and extracted with two 30-ml. portions of 5% sodium bicarbonate solution followed by 30 ml. of water. The organic phase was dried and evaporated to dryness; the aqueous solution was acidified and shaken with three 50-ml. portions of methylene chloride. The latter organic phase, on being dried over magnesium sulfate and evaporated to dryness, yielded crystalline ureido acid. The initial methylene chloride solution yielded a polyphenylalanine of about 4 residues per chain as determined by end-group measurements.

Reaction of Amines with Glycine and Sarcosine NCA's.—The reaction proper was carried out in the same manner as with phenylalanine NCA. After removal of solvent and excess amine the residuum was taken up in 10 ml. of water. One-half of this solution was stirred with *ca.* 1 g. (dry weight) of Dowex 50X8 cation exchange resin in the hydrogen form. When gas evolution had ceased, the slurry was poured onto a column of the same resin (*ca.* 5 g. dry weight, hydrogen form) and the acidic products eluted with 130 ml. of water. The eluate was concentrated to dryness under reduced pressure.

The only crystalline products isolated in the above fashion were the ureido acids derived from reaction of glycine NCA and *t*-butylamine or isopropylamine. These were weighed and then recrystallized from small quantities of water. Yields are reported in Table I and analytical data in Table III. Although less than 10 mg. of amorphous material was isolated from two experiments with sarcosine NCA, infrared spectra did not confirm the presence of ureido acids.

Other Ureido Acids.—N-(N′,N′-Diethylcarbamyl)-DL-alanine was obtained in over 70% yield by reaction of DL-alanine-N-carboxyanhydride with excess diethylamine. It was not possible to isolate DL-alanyl-N′,N′-diethylamide from the reaction products. N-(N′,N′-Diethylcarbamyl)-L-leucine, $[\alpha]^{25}D$ −5.3° (5% in ethanol), was similarly prepared from L-leucine NCA. Analytical data for these substances appear in Table III.

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(12) Infrared spectra were recorded on a Perkin-Elmer model 21 infrared spectrophotometer. Samples were prepared as pressed disks.