acid. In a three-necked flask fitted with a reflux condenser and nitrogen inlet tube were placed 45 g. (0.02 mole) of 5-heptyl-5-methylhydantoin and 104 g. (0.6 mole) of 60% sulfuric acid. The mixture was then heated at 130° for 72 hr. under a nitrogen atmosphere. The clear, straw-colored solution was then cooled and a precipitate, consisting of amino acid sulfate and unchanged hydantoin, was filtered. The precipitate was dissolved in 300 ml. of hot water, decolorized with activated charcoal, and filtered. The filtrate was cooled and adjusted to *p*H 6 with 10% aqueous ammonia, which precipitated the free amino acid. The mother liquor from the reaction mixture was diluted with 200 ml. of water, decolorized with charcoal, filtered, and the free amino acid precipitated by the addition of 10% aqueous ammonia to *p*H 6. Both crops of amino acid were combined and recrystallized, first, from 50% ethanol and then from acetic acid–water. Finally the product was dried for 24 hr. *in vacuo* at 50°; yield, 23 g., m.p., 296–300° (sealed tube).

Anal. Calcd. for $C_{10}H_{21}NO_2$: N, 7.48. Found: N, 7.33.

Method B. Base hydrolysis.[21] *2-Amino-2-methyl-5-hexenoic acid.* In a stainless steel reaction vessel were placed 30.2 g. (0.18 mole) of 5-(3-butenyl)-5-methylhydantoin, 85 g. (0.27 mole) of barium hydroxide and 485 ml. of water. The bomb was flushed with nitrogen, sealed, and heated to 165° for 30 min. After cooling to room temperature, the alkaline reaction mixture was diluted with 300 ml. of water, then aerated and heated to drive off the ammonia formed in the reaction. The solution was then acidified with concd. sulfuric acid to *p*H 1–2, the barium sulfate filtered, and the *p*H readjusted to 6 with lead carbonate. The solution was filtered free of lead sulfate and then treated with hydrogen sulfide to remove the excess lead ion. The aqueous solution was next heated to boiling, decolorized with charcoal, filtered, and the filtrate concentrated to give three crops of the free amino acid; total yield 22.5 g., m.p., 312–314°. A sample for analysis was recrystallized from 70% ethanol.

Anal. Calcd. for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78. Found: C, 58.91; H, 8.97; N, 9.86.

Several of the amino acids hydrolyzed by this method were insoluble enough in water to be isolated by concentrating the acidic solution to half volume after removing the precipitated barium sulfate and adjusting the *p*H to 6 with concd. ammonium hydroxide. The amino acid was then filtered and washed with several portions of distilled water.

Method C. Base hydrolysis. 2-Phenyl-2-p-tolylglycine. A stainless steel reaction vessel containing 22.6 g. (0.085 mole) of 5-phenyl-5-*p*-tolylhydantoin and 370 ml. of a 20% sodium hydroxide solution was flushed with nitrogen, sealed, and heated to 165° for 24 hr. The cooled reaction mixture was diluted with 1 l. of water and the *p*H adjusted to <1 with concd. hydrochloric acid. The solution was then treated with charcoal, filtered, and the *p*H readjusted to 6 with ammonium hydroxide; yield, 15 g., m.p., 244.5–245° (sealed tube).

Anal. Calcd. for $C_{15}H_{15}NO_2$: C, 74.66; H, 6.27; N, 5.81. Found: C, 74.70; H, 6.32; N, 5.75.

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(21) J. E. Livak, E. C. Britton, J. C. VanderWeele, and M. F. Murray, *J. Am. Chem. Soc.*, **67**, 2218 (1945).

[CONTRIBUTION FROM THE DEPARTMENT OF NUCLEAR MEDICINE AND BIOPHYSICS OF THE MEDICAL CENTER, UNIVERSITY OF CALIFORNIA AT LOS ANGELES, THE CHEMISTRY DEPARTMENT, FRESNO STATE COLLEGE, AND THE CHEMISTRY DEPARTMENT, LONG BEACH COLLEGE]

Behavior of Certain Pyridines, Quinolines, and Isoquinolines with Amino or Hydrazino Substituents Toward *N*-Acylamino Acids Under the Influence of Papain Catalysis

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Received October 12, 1959

3-Aminoquinoline and 3-hydrazinoquinoline have been found to undergo reactions with hippuric acid, carbobenzoxyglycine, and carbobenzoxy-L-alanine in the formation of amide-like products. Also, they both effectively resolve carbobenzoxy-DL-alanine and benzoyl-DL-alanine under papain catalysis. When benzoyl-L-alanine is used alone, however, neither of the amino-containing bases undergoes a papain-catalyzed reaction with this single antipode. A number of aminopyridines, aminoquinolines, 4-aminoisoquinoline, and 2-hydrazinoquinoline failed to react, under papain catalysis, with this same selected group of N-acylamino acids.

Papain catalysis of the formation of peptide-like linkages from N-acyl amino acids and aniline or phenylhydrazine was demonstrated in the original research of Max Bergmann and Heinz Fraenkel-Conrat.[3] Groundwork was thereby laid for a diversity of studies[4] which brought forth much informa-

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(3) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937).

(4) E. L. Bennett and C. Niemann, *J. Am. Chem. Soc.*, **70**, 2610 (1948); *J. Am. Chem. Soc.*, **72**, 1798 (1950); *J. Am. Chem. Soc.*, **72**, 1800 (1950); E. Waldschmidt-Leitz and K. Kuhn, *Z. physiol. Chem.*, **285**, 23 (1950); D. G. Doherty and E. A. Popenoe, Jr., *J. Biol. Chem.*, **189**, 447 (1951); H. B. Milne and C. M. Stevens, *J. Biol. Chem.*, **74**, 3269 (1952); S. W. Fox, M. Winitz, and C. W. Pettinga, *J. Am. Chem. Soc.*, **75**, 5539 (1953); J. P. Greenstein, *Resolutions of Racemic Alpha-Amino Acids*, Chapter IX in "Advances in Protein Chemistry," Academic Press, New York (1954); J. L. Abernethy, J. Nakamura, and Bro. Myron Collins, *J. Org. Chem.*, **23**, 586 (1958); J. L. Abernethy, M. Kientz, R. Johnson, and R. Johnson, *J. Am. Chem. Soc.*, **81**, 3944 (1959).

tion concerning the specificity of this enzyme, particularly with regard to amine structures and the ability of the enzyme to resolve a variety of *N*-acylamino acids. Usually a preference is shown by the enzyme for an L-acylamino acid in such catalysis, although this is not always the case.[4] Most of the early research was focussed on variations in the amino acids structure and the acyl radical bonded to the α-amino group. Little was done in extending the basic, amino-containing reactant to other varieties of structures. Among the few amino-containing compounds used were an aliphatic type of amine, benzylamine,[4,5] and a secondary amine, methylaniline.[5] Neither underwent a reaction with an *N*-acylamino acid. Phenylhydroxylamine[5] was also employed, but this likewise gave negative results. However, in the majority of instances studied, substituted anilines with many kinds of substituents did give rise effectively to hippuric substituted anilides.[4]

Still further extension was then made in incorporating an asymmetric center[6] in the amino-containing molecule, as in *m*-(1-hydroxyethyl)aniline. With hippuric acid the reaction went well, but the resultant hippuric *m*-(1-hydroxyethyl)anilide was racemic. Attention was then turned to hydrazides, which contain a basic amino group.[4] Effective resolutions of *N*-acylamino acids occurred during the formation of N^{α},N^{β})-diacylhydrazines. However, resolution of DL-mandelic hydrazide did not occur in its reaction with hippuric acid, nor did ethylmalonic hydrazide undergo a partial asymmetric synthesis in its reaction with hippuric acid. Racemic products resulted.

The present investigation was undertaken to incorporate nitrogen in a six-membered ring involving a resonance hybrid, rather than using a benzene ring, with amino or hydraazino groups attached to the heterocyclic nucleus. Pyridine, quinoline, and isoquinoline nuclei were used. The specific compounds chosen were 2-aminopyridine, 4-methyl-2-aminopyridine, 3-aminopyridine, 4-aminopyridine, 2-aminoquinoline, 3-aminoquinoline, 5-aminoquinoline, 6-aminoquinoline, 8-aminoquinoline, 4-aminoisoquinoline, 2-hydrazinoquinoline and 3-hydrazinoquinoline. *N*-Acylamino acids selected were hippuric acid, carbobenzoxyglycine, carbobenzoxy-L-alanine, carbobenzoxy-DL-alanine, benzoyl-L-alanine, and benzoyl-DL-alanine because of their reasonably moderate solubility.

The investigation was divided into the following phases of study: (1) Determination of the dependence of yield on *p*H for a very few reactions served as a guide for an appropriate *p*H to be utilized for an entire group of closely related syntheses. (2) Each amino-containing compound was subjected to reactions with the chosen series of *N*-acyl amino acids. Papain was the catalyst and L-cysteine the promotor at 40° and the established *p*H. Removal of the reaction product was carried out at appropriate intervals of time. (3) Tests were made for resolution when racemic *N*-acylamino acids were utilized by determination of the optical activity of the reaction product.

(5) Unreported experimental work performed at the California State Polytechnic College, San Luis Obispo, and at Fresno State College.

(6) J. L. Abernethy and Bro. Myron Collins, *J. Org. Chem.*, 1558.

EXPERIMENTAL

Activation of papain. The papain in these experiments was obtained from the Wallerstein Laboratories, New York City. Activation was carried out according to the procedure of Grassmann[7] and of Bennett and Niemann[8] with certain simplifications.[4] The resultant light tan product was crushed lightly and stored in stoppered vials in a brown bottle fitted with a screw cap and was refrigerated at 5°.

Preparation of intermediates. It was necessary to prepare some of the reactants for these studies in papain catalysis. 3-Aminopyridine was synthesized from nicotinamide by a Hofmann hypobromite reaction.[9] The preparation of 2-aminoquinoline involved a Chichibabin reaction using sodium amide and quinoline in boiling xylene.[10] Care was taken to employ freshly opened lump sodium amide,[11] which was cautiously ground to a powder under a dry nitrogen atmosphere in a dry box. The solid melting at 129–130° after recrystallization from toluene was used in the papain-catalyzed syntheses.

The preparation of 4-aminoisoquinoline[12] was effected by conversion of isoquinoline to 4-bromoisoquinoline[13] and subsequent conversion to 4-aminoisoquinoline[14] by concd. ammonium hydroxide and copper sulfate in a heated, shaking, autoclave. The 4-aminoisoquinoline was then converted to the solid hydrochloride with a stream of dry hydrogen chloride.

The synthesis of carbobenzoxyglycine, carbobenzoxy-DL-alanine, and carbobenzoxy-L-alanine was accomplished by the method of Carter, Frank, and Johnston[15] from benzyl chloroformate and the appropriate amino acids.

Dependence of yield on pH for the papain-catalyzed reactions between 3-aminoquinoline and hippuric acid to form 3-hippuramidoquinoline. It was necessary to perform exploratory experiments to find a wide range of *p*H values over which there was sufficient solubility of both hippuric acid and 3-aminoquinoline and also insolubility of 3-hippuramidoquinoline to determine satisfactorily the dependence of yield on *p*H. For the *p*H range from 4.0 to 6.0, with 0.5 unit increments of *p*H for the study and 0.01 mole each of hippuric acid and 3-aminoquinoline in 125 ml. of total buffered solution, a precipitate was given only at *p*H 5.5. When the quantities of hippuric acid and 3-aminoquinoline were increased to

(7) W. Grassmann, *Biochem. Z.*, **279**, 131 (1935).

(8) E. L. Bennett and C. Niemann, *J. Am. Chem. Soc.*, **72**, 1789 (1950).

(9) C. F. H. Allen and C. N. Wolf, *Org. Syntheses*, **30**, 3 (1950).

(10) D. A. Shirley, *Preparation of Organic Intermediates* John Wiley and Sons, New York (1951), p. 16.

(11) Fisher Scientific Company Chemicals, lump sodium amide.

(12) D. A. Shirley, *Preparation of Organic Intermediates*, John Wiley and Sons, New York (1951), p. 50.

(13) F. W. Bergstrom and J. H. Rodda, *J. Am. Chem. Soc.*, **62**, 3031 (1940).

(14) D. A. Shirley, *Preparation of Organic Intermediates*, John Wiley and Sons, New York (1951), p. 11.

(15) H. E. Carter, R. L. Frank, and H. W. Johnston, *Org. Syntheses*, **Coll. Vol. III**, 158 (1955).

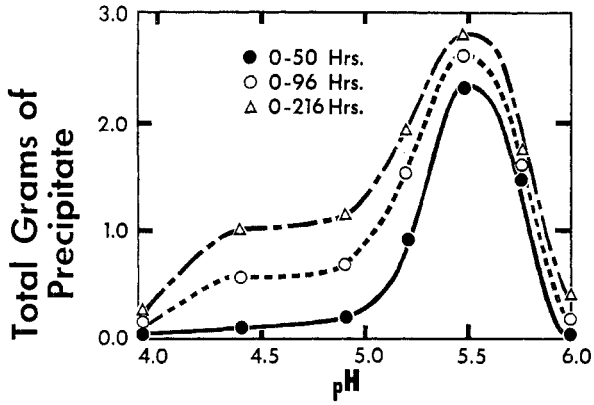


Fig. 1. Dependence of yield on *p*H for the papain-catalyzed formation of 3-hippuramidoquinoline from hippuric acid and 3-aminoquinoline

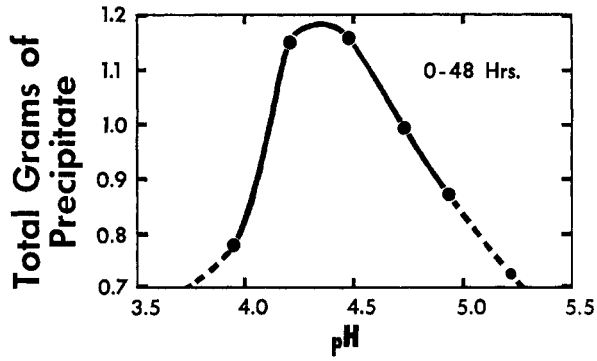


Fig. 2. Dependence of yield on *p*H for the formation of hippuric 3-quinolylhydrazide from hippuric acid and 3-hydrazinoquinoline

0.02 mole each for this same volume of solution, a considerable amount of undissolved reactant remained at the higher pH values. Satisfactory solution was brought about for the entire *p*H range studied by using 0.0200 mole of hippuric acid and 0.0100 mole of 3-aminoquinoline, 0.500 g. of L-cysteine hydrochloride, 0.500 g. of activated Wallerstein papain, and enough buffer to make 125 ml. total of buffered solution. After mixing and filtering, the solutions were adjusted to the exact *p*H desired, with the aid of a *p*H meter. The solutions were incubated at 40° and the precipitates were collected at the end of 50, 96, and 216 hr., with readjustment of the *p*H to its original value after each filtration. Precipitates were dried and weighed. Results are plotted in Fig. 1. Total yields in percent for 216 hr. of incubation were: *p*H 6.01, 13.1%; *p*H 5.75, 55.9%; *p*H 5.47, 93.0%; *p*H 5.22, 66.1%; *p*H 4.92, 37.4%; *p*H 4.40, 33.4%; *p*H 3.95, 7.1%.

Dependence of yield on pH for the reaction between 3-hydrazinoquinoline and hippuric acid to form hippuric 3-(quinolyl)-hydrazide. Preliminary experiments showed that effective results could be obtained by employing 1.161 g. (0.00500 mole) of 3-hydrazinoquinoline, 3.583 g. (0.0200 mole) of hippuric acid, 0.500 g. of L-cysteine hydrochloride, and 0.500 g. of activated Wallerstein papain in sufficient buffer to make 125 ml. of solution. Filtration was carried out before incubation was started. Some insoluble substrate was noted. Incubation was conducted at 40° followed by collection of the precipitates at the end of 48 hr. for each of the *p*H values used. The results are shown graphically in Fig. 2. Percent yields at the end of 48 hr. were: *p*H 3.95, 22.8%; *p*H 4.23, 34.0%; *p*H 4.49, 34.2%; *p*H 4.73, 29.7%; *p*H 4.95, 25.6%.

Experiments with aminopyridines. A number of experiments were tried with 2-aminopyridine, 3-aminopyridine, and 4-methyl-2-aminopyridine. Both hippuric acid and carbobenzoxyglycine were used as the amido acids, with L-cysteine hydrochloride as the promotor and activated Wallerstein papain as the catalyst. The *p*H was varied from about 3.5 to 6.0 at increments of about 0.5 *p*H units. After 5 days or more, no evidence was given for a reaction in any of the situations studied, when incubation was carried out at 40°.

Experiments using 2-aminoquinoline, 5-aminoquinoline, 8-aminoquinoline and 4-aminoisoquinoline with hippuric acid, carbobenzoxyglycine, carbobenzoxy-DL-alanine and carbobenzoxy-L-alanine. These experiments were conducted over a range of *p*H values from 4 to 6 at 0.5 units of *p*H. In all cases the amino compounds were quite insoluble at high *p*H values. At the end of 264 hr. of incubation no precipitate of product had been obtained when incubated at 40°, with activated Wallerstein papain as the catalyst and L-cysteine hydrochloride as the promotor.

Experiments with 3-aminoquinoline and hippuric acid, carbobenzoxyglycine, carbobenzoxy-L-alanine, carbobenzoxy-DL-alanine, benzoyl-L-alanine and benzoyl-DL-alanine. All of these experiments were carried out in 125 ml. total buffered solution at *p*H 5.5 and incubated at 40°. In each case 0.0100 mole of 3-aminoquinoline was employed with 0.500 g. of activated Wallerstein papain and 0.500 g. of L-cysteine hydrochloride. The amounts of *N*-acylamino acids employed for the separate reactions were: 0.0150 mole of hippuric acid; 0.0150 mole of carbobenzoxyglycine; 0.0150 mole of carbobenzoxy-L-alanine; 0.0150 mole of benzoyl-L-alanine; 0.0300 mole of carbobenzoxy-DL-alanine; 0.0300 mole of benzoyl-DL-alanine. Precipitates were collected at the end of 18, 44, and 264 hr. of incubation and were dried and weighed. After recrystallization from an ethanol and water mixture, melting points were determined. Following each filtration, the filtrate was readjusted to *p*H 5.5 before incubation was continued. Specific rotations were determined in approximately 2% solutions in pyridine. Results are given in Table I. No product was given with benzoyl-L-alanine.

Experiments between 3-hydrazinoquinoline and hippuric acid, carbobenzoxyglycine, benzoyl-L-alanine, benzoyl-DL-alanine, carbobenzoxy-L-alanine, and carbobenzoxy-DL-alanine. These reactions were all conducted at *p*H 4.5 and 40°. 3-Hydrazinoquinoline dihydrochloride (0.0100 mole) was employed with 0.0300 mole of hippuric acid, carbobenzoxyglycine, carbobenzoxy-L-alanine, or benzoyl-L-alanine or with 0.0600 mole of carbobenzoxy-DL-alanine or benzoyl-DL-alanine. A total of 250 ml. of buffered solution was utilized with 1.000 g. of activated Wallerstein papain and 1.000 g. of L-cysteine hydrochloride. Results are tabulated in Table II. No product was given with benzoyl-L-alanine. Precipitates were collected at the end of 18, 44, and 168 hr., dried and weighed. The filtrates were readjusted to *p*H 4.5 before incubation was continued. As zero rotation was shown in each case in pyridine, rotations were determined in chloroform or methanol.

Rotations of α-(benzyloxycarbonylamino)propionic 3-quinolylhydrazide and α-benzamidopropionic 3-quinolylhydrazide in pyridine. Both of these 3-quinolylhydrazides displayed zero rotation when dissolved in pyridine, which suggested possible racemization. α-(Benzyloxycarbonylamino)propionic 3-quinolylhydrazide showed $[\alpha]_D^{25°} = -25.3°$ in chloroform. When the chloroform was removed by evaporation and the solid was dissolved in pyridine, zero rotation was obtained. When the pyridine was then evaporated and this solid was redissolved in chloroform, zero rotation was obtained.

On the other hand, α-benzamidopropionic 3-quinolylhydrazide exhibited $[\alpha]_D^{25°} = +40.0°$ in methanol. Upon removal of the methanol and redissolving the solid in pyridine, zero rotation was shown. Removal of the pyridine and

TABLE I

REACTIONS OF 3-AMINOQUINOLINE WITH *N*-ACYLAMINO ACIDS AT *p*H 5.5 AND 40°

Amido Acids[a]	Product	M.P.[b]	$[\alpha]_D^{25°}$ 2% in pyridine	Yield, g. 0–18 Hr.	Yield, g. 18–44 Hr.	Yield, g. 44–264 Hr.	N Calcd.	N Found
HA	3-Hippuramidoquinoline	218–220°		0.997	0.446	0.631	13.76	13.76
CBG	3-Carbobenzoxyglycylamidoquinoline	237–238°		2.707	0.245	0.369	12.53	12.49
C-L-A	3-Carbobenzoxy-L-alanylamidoquinoline	179–180°	−77.9°	3.262	0.762	0.209	12.61	12.79
C-DL-A	3-Carbobenzoxyalanylamidoquinoline	180–182°	−73.9°	1.326	0.797	0.151	12.61	12.62
B-DL-A	3-Benzoylalanylamidoquinoline	204–205°	−46.55°	2.533	0.264	0.125	13.16	13.15

[a] Hippuric acid (HA); carbobenzoxyglycine (CBG); carbobenzoxy-L-alanine (C-L-A); carbobenzoxy-DL-alanine (C-DL-A); benzoyl-L-alanine (B-L-A), no reaction; benzoyl-DL-alanine (B-DL-A). [b] Recrystallized from ethanol and water.

TABLE II

REACTIONS OF 3-HYDRAZINOQUINOLINE WITH *N*-ACYLAMINO ACIDS AT *p*H 4.5 AND 40°

Amido Acid[a]	Product	M.P.[b]	$[\alpha]_D^{25°}$ 2% in solvent	Yield, g. 0–18 Hr.	Yield, g. 18–44 Hr.	Yield, g. 44–264 Hr.	N Calcd.	N Found
HA	Hippuric 3-quinolylhydrazide	213–214°		1.320	0.019	0.040	17.49	17.69
CBG	Benzyloxycarbonylaminoacetic 3-quinolylhydrazide	179–180°		1.290	0.020	0.000	16.00	15.99
C-L-A	L-α-(Benzyloxycarbonylamino)propionic 3-quinolylhydrazide	197–198°	−25.3° in $CHCl_3$	1.197	0.183	0.000	15.38	15.32
C-DL-A	α-(Benzyloxycarbonylamino)propionic 3-quinolylhydrazide	195–196°	−25.1° in $CHCl_3$	1.511	0.273	0.019	15.38	15.36
B-DL-A	α-Benzamidopropionic 3-quinolylhydrazide	228–230°	+40.0° in CH_3OH	1.928	0.466	0.086	16.76	16.61

[a,b] See footnotes for Table I.

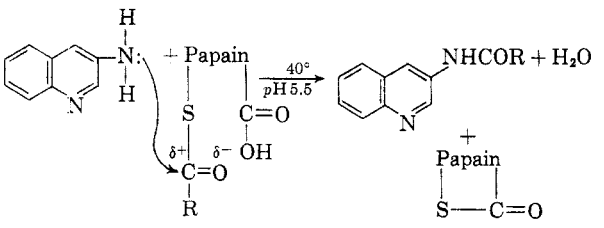
redissolving in methanol gave substantially the same rotation that was given before in methanol.

DISCUSSION

3-Aminoquinoline and 3-hydrazinoquinoline were two compounds containing the quinoline nucleus and, incorporating a basic amino group in their structures, that underwent reactions with hippuric acid, carbobenzoxyglycine, carbobenzoxy-DL-alanine, carbobenzoxy-L-alanine, and benzoyl-DL-alanine. No products were formed when 2-aminopyridine, 3-aminopyridine, 4-methyl-2-aminopyridine, 2-aminoquinoline, 5-aminoquinoline, 8-aminoquinoline, and 4-aminoisoquinoline were each studied with hippuric acid, carbobenzoxyglycine, carbobenzoxy-DL-alanine, and carbobenzoxy-L-alanine. Solubilities of reactants and also of products play a determining role. Relative basicities of amino groups are important contributing factors. Resonance withdrawal of the electron pair on amino nitrogen, particularly when enhanced by the heterocyclic nitrogen, would be significant in reducing the basicity of amino nitrogen. Besides this, coulombic attraction of the hetero nitrogen atom for this electron pair on amino nitrogen would reduce the availability of the pair and therefore the basic properties of the amido group.

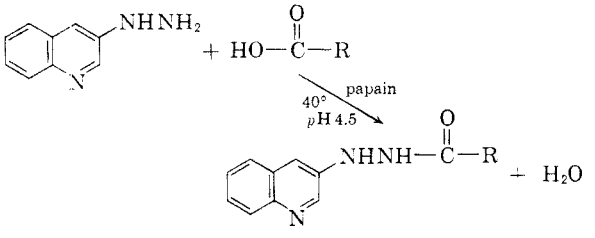
The failure of any of the aminopyridines to respond might, in part, also be attributed to relatively high solubilities of anticipated products of reaction. Generally speaking, one of the driving forces of these reactions is the insolubility of the products, which can keep the essentially steady-state equilibria disturbed in favor of these desired substances.

3-Aminoquinoline contains an additional benzene ring, which would decrease the solubility of the reaction product. It has an appropriately situated amino radical for enhancing its basicity and ability to react with the papain-amido acid substrate intermediate.



The possibility of assistance in the reaction by an imidazolyl radical of an L-histidine residue, if spaced appropriately between an L-cysteine residue and an L-glutamic acid residue of the papain polypeptide chain, has been discussed in detail in connection with previous work.[6,7] These reactions of 3-aminoquinoline show that an amino substituent can be bonded to a heterocyclic ring and undergo a reaction similar to an amino group bonded to a benzene ring. It should be pointed out that an investigation of the dependence of yield on pH for the reaction product of the reaction between hippuric acid and 3-aminoquinoline showed a relatively high optimum *p*H of about 5.5, for the conditions employed.

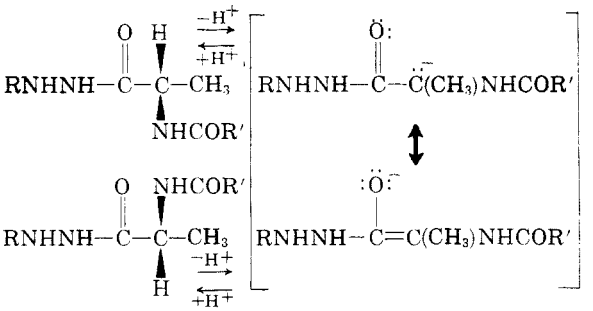
In contrast with this, 3-hydrazinoquinoline undergoes a reaction with hippuric acid at an optimum of about 4.5.



This is the first hydrazino group bonded to something besides a benzene ring or a carbonyl group that has been demonstrated to take part in such a reaction. 4-Aminoisoquinoline did not undergo a reaction with any of the acylated amino acids of this investigation.

A very peculiar situation has consistently arisen with benzoyl-DL-alanine and benzoyl-L-alanine throughout these and previous experiments.[4,6] In all instances where a reaction did occur with benzoyl-DL-alanine and appropriate amino-containing compounds under papain-catalysis, resolution of the racemic *N*-acylamino acid resulted. When benzoyl-L-alanine was used alone no reaction took place. This is not readily explained on the basis of the compounds. Perhaps some inhibitor is present in the benzoyl-L-alanine. Further investigation is planned for this puzzling situation.

The zero rotation of α - (benzyloxycarbonylamino)propionic 3-quinolylhydrazide α-benzamidopropionic 3-quinolylhydrazide in pyridine suggested that racemization might have occurred in this basic solvent. This explanation may apply to



the α-benzyloxycarbonyl derivative (see Experimental) but cannot apply to the α-benzamido derivative, which gave a rotation in methanol, both before and after the measurement of (zero) rotation in pyridine.

Acknowledgment. Funds for this research were generously provided by the Fresno County Heart Association, the California Heart Association, the Sigma Xi Society and RESA, and the Research Corporation. Dr. Kendall Holmes, Dr. Robert D. Beech, Dr. Clell Gray, and Mrs. Joyce Richardson of the Fresno County Heart Association, and Dr. John J. Sampson, Dr. Robert H. Maybury, and Miss Phyllis Hecker of the California Heart Association were instrumental in securing Heart Association grants. Mr. William Woods of the Research Corporation was particularly helpful in working with state college administrators in securing permission to carry out the research before state policy was established.

(7) T. C. Bruice, *J. Am. Chem. Soc.*, 81, 5444 (1959).

LOS ANGELES CALIF.