

under an atmosphere of nitrogen for 20 hours. It was extracted with water and then with dilute sulfuric acid. The acid extract was made basic with dilute potassium hydroxide and extracted with ether. This ether extract was dried over sodium sulfate and the product was precipitated by ethereal hydrogen chloride; 1.5 g. (60%). The hydrochloride (m.p. 219–220°) was converted to the free base which melted at 87–88°.

*Anal.* Calcd. for  $C_{14}H_{19}NO$ : N, 5.59. Found: N, 5.74.

**Aluminum isopropoxide reduction** of a solution of 2.5 g. of the hydrochloride (0.01 mole) and 5.0 g. (0.025 mole) of the reagent was carried out in isopropyl alcohol under reflux for 1.5 hr. The excess solvent was distilled under reduced pressure, dilute sodium hydroxide was added and the solution was extracted with ether. The ether was extracted with dilute hydrochloric acid and the free amino alcohol was liberated by the addition of dilute sodium hydroxide; m.p. 164–166° (a mixture melting point with authentic Vc gave no depression); yield 1.0 g. (46%).

**erythro-2-[N-Ethyl-N-( $\beta$ -hydroxyethyl)-amino]-1,2-diphenylethanol (VII)** (known<sup>14</sup>), m.p. 82–84° (base, recrystallized from dil. ethanol), on recovery from the at-

tempted Oppenauer oxidation gave a sample of hydrochloride of m.p. 182–183°. This product gave no mixture melting point depression when admixed with starting material of m.p. 176–178°. The base when isolated crystallized in a polymorphic form of m.p. 91–92°. The original base, of m.p. 82–84° on fusion and resolidification, subsequently melted at 91–92°; no melting point depression was observed upon admixture with the recovered sample of this melting point.

*Anal.* (base, m.p. 91–92°). Calcd. for  $C_{18}H_{23}NO_2$ : C, 75.76; H, 8.12. Found: C, 76.09; H, 8.34. *Anal.* (hydrochloride, m.p. 181–182°). Calcd. for  $C_{18}H_{23}NO_2 \cdot HCl$ : C, 67.59; H, 7.47. Found: C, 66.96; H, 7.34.

**threo-2-[N-Ethyl-N-( $\beta$ -hydroxyethyl)-amino]-1,2-diphenylethanol (VII)** was prepared by heating under reflux a mixture of 5 g. of *cis*-stilbene oxide, 0.6 g. of ethylethanolamine and 2.9 g. of its hydrochloride for 2 hours. After treatment with water, extraction of the base with ether and crystallization from dilute ethanol, 4.1 g. (69%) was obtained, m.p. 101–102°.

*Anal.* Calcd. for  $C_{18}H_{23}NO_2$ : C, 75.76; H, 8.12. Found: C, 75.60; H, 8.45.

(14) Lutz, Freck and Murphey, *ibid.*, **70**, 2015 (1948).

CHARLOTTESVILLE, VA.

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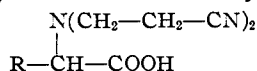
[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY<sup>1</sup>]

## Cyanoethylation of $\alpha$ -Amino Acids. II. Dicyanoethyl and Tricyanoethyl Derivatives<sup>2</sup>

By L. L. MCKINNEY, E. H. UHING, E. A. SETZKORN AND J. C. COWAN

A series of N-dicyanoethyl derivatives of  $\alpha$ -amino acids has been prepared by treating aqueous solutions of the alkali metal salts with acrylonitrile. N-Dicyanoethyl derivatives of the following amino acids are reported: glycine, DL-alanine, DL-valine, L-leucine, DL-methionine, L-tyrosine, DL-aspartic acid and L-glutamic acid. The difficulty with which the second cyanoethyl group added increased in the order: glycine, alanine, aspartic acid, glutamic acid, leucine, methionine, valine, tyrosine. Tyrosine reacted very slowly in aqueous alkaline solution to form the O-cyanoethyl derivative. The dicyanoethyl derivatives exhibited true melting points and were soluble in organic solvents. The second cyanoethyl group was labile to heat, alkali and acid.

A previous communication<sup>3</sup> described the preparation of monocyanoethyl derivatives of  $\alpha$ -amino acids. The present paper describes the preparation of dicyanoethyl derivatives of the type



Tricyanoethyl derivatives of histidine and tyrosine are also described.

and aspartic acid were readily prepared by heating aqueous solutions of the alkaline salts of the amino acids with 2 equivalents of acrylonitrile. Preparation of the corresponding derivatives of valine, leucine, methionine, tyrosine and glutamic acid required prolonged refluxing.

All the N-dicyanoethyl derivatives were soluble in organic solvents and exhibited true melting points (see Table I) in contrast to the monocyano-

TABLE I

N-DICYANOETHYL DERIVATIVES OF  $\alpha$ -AMINO ACIDS

Amino acid	M.p., °C.	Solubility, <sup>b</sup> g./100 ml. solvent			Molecular formula	Carbon, %		Hydrogen, %		Nitrogen, %	
		Water	Ether	Acetone		Calcd.	Found	Calcd.	Found	Calcd.	Found
Glycine	77.8–78.8	52	0.7	34	$C_8H_{11}O_2N_2$	53.0	52.8	6.11	5.96	23.2	23.2
DL-Alanine	75.5–76.8	13	1.3	61	$C_9H_{13}O_2N_2$	55.4	55.5	6.71	6.50	21.5	21.4
DL-Valine	54–55	2.3 <sup>c</sup>	>100	>100	$C_{11}H_{17}O_2N_2$	59.2	59.8	7.67	7.75	18.8	18.8
L-Leucine	64–65	1.7 <sup>d</sup>	29	>100	$C_{12}H_{19}O_2N_2$	60.7	60.1	8.07	8.02	17.7	17.7
DL-Methionine	65–66	1.2 <sup>e</sup>	4.3	>140	$C_{11}H_{17}O_2N_2S$	51.7	51.8	6.71	6.66	16.5	16.5
L-Tyrosine	123–124	0.6 <sup>f</sup>	0.7	43	$C_{15}H_{17}O_3N_2$	62.7	62.5	5.96	5.83	14.6	14.5
DL-Aspartic acid	136–137	0.7 <sup>g</sup>		7.5 <sup>h</sup>	$C_{10}H_{13}O_4N_2$	50.1	50.0	5.48	5.11	17.5	17.5
L-Glutamic acid <sup>a</sup>	71.5–72.8	15	1.1	>100	$C_{11}H_{17}O_5N_2$	48.7	49.0	6.32	6.29	15.5	15.5

<sup>a</sup> Monohydrate. <sup>b</sup> Room temperature, 25–27° except as shown otherwise. <sup>c</sup> At 5°. <sup>d</sup> 2.8 g. at 80°. <sup>e</sup> 0.6 g. at 7°. <sup>f</sup> 11 g. at 100°. <sup>g</sup> 100 g. at 80°. <sup>h</sup> At 50°.

### N,N-Dicyanoethyl derivatives of glycine, alanine

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Presented before the Division of Biological Chemistry at the 117th Meeting of The American Chemical Society, Chicago, Illinois, September 3–8, 1950.

(3) McKinney, *et al.*, *THIS JOURNAL*, **72**, 2599 (1950).

ethyl derivatives<sup>3</sup> which retained the dipolar character of the amino acid.

The electrometric titration curves for DL-alanine, DL-methionine and L-glutamic acid and their mono- and di-cyanoethyl derivatives are shown in Fig. 1. Curves 1 in each case are for the unreacted amino acid and curves 2 are for the monocyanoethyl

derivatives. It is apparent that the  $-\text{NH}_2\text{R}^+$  group is a stronger acid than the  $-\text{NH}_3^+$  group. The broken line portion of curves 3 represent no more than the titration of hydrochloric acid.

Evidence for the titration of free hydrochloric acid is based on the properties of dicyanoethylmethionine hydrochloride. This compound was prepared under anhydrous conditions and when added to water, an oil separated which was extracted with ether and proved to be the free dicyanoethylmethionine. The introduction of two cyanoethyl groups weakens the basic character of the amino group to the point where it will no longer form a stable hydrochloride. It should be noted that curves 3 in Fig. 1A and 1B are similar to curves obtained by titrating equal moles of hydrochloric and acetic acid with sodium hydroxide. The same interpretation is applied to curve 3 in Fig. 1C.

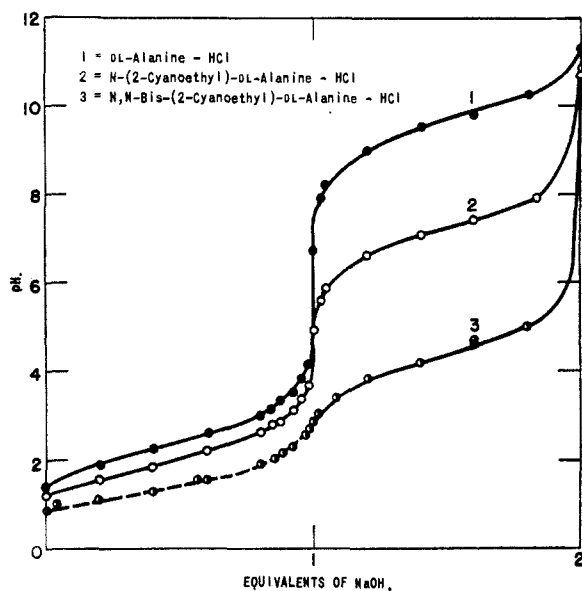


Fig. 1A.

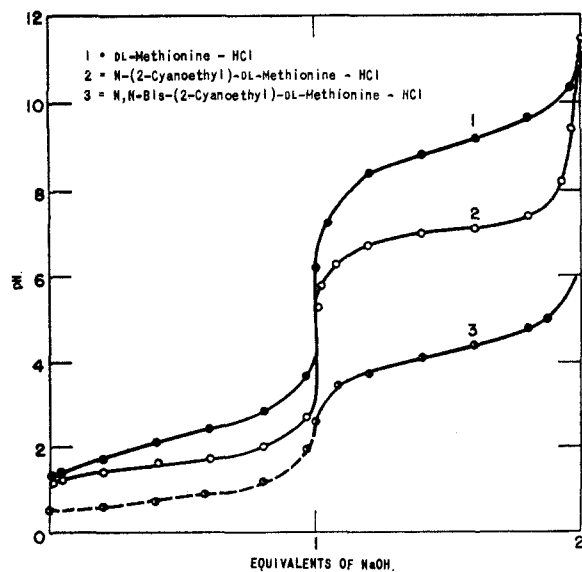


Fig. 1B.

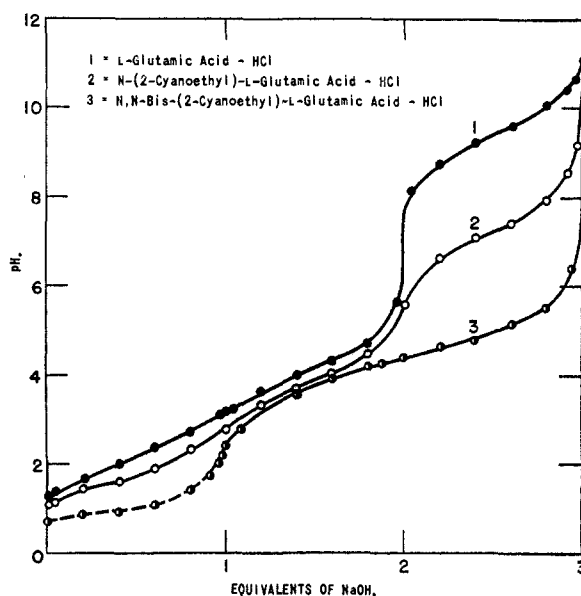


Fig. 1C.

Fig. 1.—Titration curves showing the effect of substitution on the  $\alpha$ -amino group. The broken line portion of curves 3 represent unstable hydrochlorides.

In the preparation of *N,N*-dicyanoethyltyrosine, it was necessary to use excess acrylonitrile because of losses during prolonged refluxing. In addition to the *N,N*-dicyanoethyl derivative, a 3.5% yield of a compound believed to be *N,N*-bis-(2-cyanoethyl)-*O*-(2-cyanoethyl)-tyrosine was obtained. The compound melted lower and had greater solubility in organic solvents than the di-adduct. It gave a negative Millon test, no color with the Folin phenol reagent, and its analysis corresponded to tricyanoethyl derivatives.

When an aqueous solution of sodium histidinate was heated with 4 equivalents of acrylonitrile, a product with an analyses of a tricyanoethyl derivative was obtained. It melted with decomposition, and was insoluble in organic solvents, indicating that only one cyanoethyl group had been added to the  $\alpha$ -amino group. The titration curve indicated the presence of a hydrogen on the  $\alpha$ -amino group.

The optical rotation of the *N*-dicyanoethyl derivatives of *L*- $\alpha$ -amino acids are recorded in Table II. The optical activity of these derivatives may be compared with those of the monocyanoethyl derivatives and the free amino acids which were determined under the same conditions.<sup>3</sup>

TABLE II  
OPTICAL ROTATIONS OF *L*-AMINO ACIDS AND THEIR MONO- AND DI-CYANOETHYL DERIVATIVES<sup>a</sup>

Amino acid	Free amino acid	$[\alpha]_D^{25}$ Mono-adduct	$[\alpha]_D^{25}$ Di-adduct
<i>L</i> -Leucine <sup>b</sup>	+16.1	+25.9	-9.3
<i>L</i> -Glutamic acid <sup>b</sup>	+31.8	+25.1	-68.9
<i>L</i> -Tyrosine <sup>c</sup>	-14.35	+12.37	-60.4

<sup>a</sup> Concentrations of 0.1 *M*. <sup>b</sup> Solvent 0.4 *N* HCl. <sup>c</sup> Solvent 0.205 *N* NaOH.

Whereas the monocyanoethyl derivatives were relatively stable to heat and hydrolyses,<sup>3</sup> the dicyano-

ethyl derivatives were labile. For example, when *N,N*-bis-(2-cyanoethyl)-alanine was heated to 140–150°, gas was evolved and the residue solidified. It melted, with decomposition, at approximately 250° which corresponds to the melting point of the monocyanoethyl derivative. Attempts to hydrolyze with alkali resulted in recovery of the mono- but not the di-propionic acid derivative. Attempts to convert the dicyanoethyl derivatives to esters with ethanolic hydrogen chloride resulted in the recovery of ethyl acrylate and esters of the monocyanoethyl derivative.

### Experimental

**Dicyanoethyl Derivatives of  $\alpha$ -Amino Acids.**—Each amino acid (1 mole) was dissolved<sup>4</sup> in water containing sodium hydroxide (or potassium hydroxide) equivalent to the carboxyl groups. The temperature was kept below 30° to prevent deamination. Acrylonitrile (2.1 to 2.2 moles) was added and the mixture was shaken in an erlenmeyer flask. After standing overnight at room temperature, the mixture was heated in the flask fitted with a reflux condenser as follows to complete the reaction:

Amino acid	Temp., °C.	Time, hr.
Glycine	50	2
DL-Alanine	50	4
DL-Valine	Reflux	20
L-Leucine	Reflux	12
DL-Methionine	Reflux	19
L-Tyrosine	Reflux	28
DL-Aspartic acid	Reflux	4
L-Glutamic acid	60	20

The reaction was followed by removing an aliquot, evaporating unreacted acrylonitrile and determining Kjeldahl nitrogen.<sup>3</sup> The aqueous reaction mixture was acidified with an amount of hydrochloric acid equivalent to the alkali used and the derivatives were isolated as follows (analyses are shown in Table I).

***N,N*-Bis-(2-cyanoethyl)-glycine.**—The aqueous solution was evaporated to dryness and the sirupy residue taken up with acetone. After filtering off the sodium chloride, the acetone was distilled off and the product dissolved in 200 ml. of isopropyl alcohol. After standing at 5° for 2 to 5 days, crystallization was complete (90% or 163 g.). Recrystallization was effected from isopropyl alcohol-chloroform with a recovery of 93% (151 g.). The sodium salt was isolated by crystallizing from 90% hot ethanol. The crystals melted at 181–182° and decomposed at 240–250°.

***N,N*-Bis-(2-cyanoethyl)-DL-alanine.**—The acidified solution was allowed to stand overnight at 5° and the crystals were collected on a Büchner funnel (175 g.). The filtrate was evaporated to dryness and the residue extracted with chloroform, which on evaporation yielded an additional 30 g. of the crude product for a total yield of 97%. The crude material on recrystallization from 400 ml. of hot water yielded 175 g. (90%).

***N,N*-Bis-(2-cyanoethyl)-DL-valine.**—The oil which settled after acidification was separated. The aqueous layer was extracted with ether and the extract combined with the oil. The crude product (172 g., 84%) was obtained by adding petroleum ether and cooling on Dry Ice. Recrystallization from ether-petroleum ether by cooling on Dry Ice gave a recovery of 80%. It was necessary to filter the crystals cold because they melted when warmed to room temperature. Once dry, the crystals showed no tendency to revert to an oil until water was added.

An alternate method of purification was to shake the oily residue in hot water and allow it to stand at 5° until it crystallized (several days). Recovery was 97%.

***N,N*-Bis-(2-cyanoethyl)-L-leucine.**—The aqueous solution was evaporated to dryness under reduced pressure. The residue was extracted with ether and the crude product crystallized upon air evaporation of the ether at room temperature (224 g., 95%). Recrystallization from ether-

petroleum ether gave an over-all yield of 85% (202 g.) of the pure derivative.

***N,N*-Bis-(2-cyanoethyl)-DL-methionine.**—The oil which separated from the aqueous mixture upon acidification was removed. The aqueous phase was evaporated to dryness and the residue extracted with acetone. The acetone was evaporated and the residual oil combined with the original oily fraction. The oil was dissolved in 400 ml. of ether and cooled on Dry Ice (232 g., 91%). After recrystallizing twice from ether an over-all yield of 82% (209 g.) of the pure derivative was obtained.

The hydrochloride was prepared by dissolving the free acid in ether and passing in dry hydrogen chloride. The precipitate was dried over sodium hydroxide: N, found, 14.1; calcd. for the hydrochloride, 14.4. It was very hygroscopic and decomposed at 53–55°. When dissolved in water an oily layer formed immediately which was extracted with ether and crystallized giving the free acid (91% recovery). Electrometric titration of the aqueous phase indicated free hydrochloric acid and was identical with the broken part of curve 3, Fig. 1C.

***N,N*-Bis-(2-cyanoethyl)-DL-aspartic Acid.**—The product failed to crystallize from the acidified solution which contained salt. Therefore, the solution was evaporated to dryness under reduced pressure. The residue was ground in a mortar, suspended in ice-water, filtered and washed free of chloride ions. The crude yield was 88% and after recrystallizing twice from hot water the over-all yield was 73% (174 g.).

***N,N*-Bis-(2-cyanoethyl)-L-glutamic Acid.**—The reaction mixture was kept cold during acidification to prevent formation of oils. After standing overnight at 5°, the crystals (231 g.) were collected on a Büchner funnel and washed free of chloride. The filtrate and washings were evaporated for a second crop of 9 g. giving a total yield of 88.4% of the crude product which proved to be the monohydrate. The monohydrate was extracted with 400 ml. of acetone and 350 ml. of water was added. Air was passed through the solution to evaporate the acetone, and a yield of 226 g. (84%) of monohydrate crystals was obtained. The neutral equivalent was 270 (calcd. for the monohydrate 271). The water of hydration was lost on drying in vacuum at 100°.

### Tricyanoethyl Derivatives

**Tricyanoethyl-L-tyrosine.**—The chloroform solution obtained on trituration of crude *N,N*-bis-(2-cyanoethyl)-L-tyrosine, described above, yielded 12 g. of material which on recrystallization three times from chloroform-ether gave a product which had the analysis of tricyanoethyltyrosine.

*Anal.* Calcd. for  $C_{13}H_{19}O_3N_4$ : C, 63.5; H, 5.88; N, 16.5. Found: C, 63.6; H, 5.68; N, 16.3.

The crystals melted at 90–91° and gave a negative Millon test.<sup>5</sup> They yielded no color with the Folin phenol reagent.<sup>6</sup>

**Tricyanoethyl-L-histidine.**—One-fourth mole (51.9 g.) of histidine-monohydrochloride-monohydrate was stirred into 150 ml. of cold water containing 0.5 mole of sodium hydroxide. Four equivalents (1 mole) of acrylonitrile was added and the mixture allowed to stand overnight at room temperature. The flask was equipped with a condenser and the mixture was heated at 50 to 60° for 2 hours. Hydrochloric acid (0.25 mole) was added. The product crystallized from the acidified mixture upon standing at 5° overnight. It was insoluble in organic solvents and 5% soluble in cold water. After washing with cold water, the crude derivative was recrystallized from hot water (39 g., 50%). An additional 20% of slightly less pure product was obtained by concentrating the filtrate and washings and crystallizing from water. The crystals melted at 184–186° with decomposition, and gave a neutral equivalent of 300 in water and 310 in the presence of formaldehyde (calcd. 314).

*Anal.* Calcd. for  $C_{13}H_{19}O_3N_4$ : C, 57.3; H, 5.73; N, 26.7. Found: C, 56.8; H, 5.91; N, 26.3.

**Titration Curves.**—Electrometric titrations were carried out with a glass electrode. The free amino acid or its de-

(5) An excess of Millon reagent was used because of the nitrile groups which tied up the mercury in the reagent. This effect was demonstrated with *N,N*-bis-(2-cyanoethyl)-tyrosine which gave a negative test with a normal amount of reagent; however, when an excess of reagent was employed, the test was positive.

(6) Folin and Ciocalteu, *J. Biol. Chem.*, **73**, 627 (1927).

(4) With tyrosine, a suspension of the monosodium salt was used.

rivative (0.025 mole) was placed in water, 0.025 mole of hydrochloric acid added, and made up to 50 ml. volume. Titrations were made with one normal sodium hydroxide so that 25 ml. of the base was equal to one equivalent of the group being titrated. This procedure gave curves for amino acids of approximately the correct  $pK$  values in the acid range but slightly low in the basic range because of the dilution factor.

**Acknowledgment.**—The authors are indebted

to C. H. Van Etten and Mary B. Wiele of the microanalytical section of this Laboratory for carbon and hydrogen analyses and Dumas nitrogen determinations and to Dr. F. R. Senti of this Laboratory and Dr. Murray Halwer of the Eastern Regional Research Laboratory for assistance in interpreting the titration curves.

PEORIA, ILL.

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[CONTRIBUTION NO. 1444 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

## The Papain-catalyzed Synthesis of Acyl-D- and L-Phenylalanylphenylhydrazides from a Series of Enantiomorphic Pairs of Acylated Phenylalanines

BY WALTER HARRY SCHULLER AND CARL NIEMANN<sup>1</sup>

The first intimation that the papain-catalyzed synthesis of acylated  $\alpha$ -amino acid phenylhydrazides<sup>2</sup> from the corresponding acylated  $\alpha$ -amino acids and phenylhydrazine is not necessarily restricted to L-isomers was obtained by Bennett and Niemann<sup>3</sup> who showed that the reaction of carbobenzoxy-*o*-fluoro-DL-phenylalanine with phenylhydrazine in the presence of cysteine activated papain gave in addition to the expected carbobenzoxy-*o*-fluoro-L-phenylalanylphenylhydrazide, considerable amounts of the D-isomer. Further work by these investigators,<sup>4</sup> principally with acylated DL-phenylalanines, indicated that the nature of the acyl group is an important factor in determining the stereochemical specificity of the reaction. *E.g.*, with a series of five acylated DL-phenylalanines two, *i.e.*, acetyl- and benzoyl-, appeared to give only the L-phenylhydrazides,

and L-phenylhydrazides. It should be noted that Milne and Stevens<sup>5</sup> have shown that carballyloxy-DL-leucine reacts with phenylhydrazine in the presence of cysteine activated papain to give both D- and L-phenylhydrazides and that the D-phenylhydrazide is similarly formed from carballyloxy-D-leucine and phenylhydrazine.

In this investigation a series of six enantiomorphic pairs of acylated phenylalanines were allowed to react individually at 40° and pH 4.6, with phenylhydrazine in the presence of cysteine activated papain and the extent of hydrazide formation determined by isolation of the corresponding phenylhydrazides. It will be seen from the data obtained in these experiments and summarized in Tables I and II that with the individual enantiomorphs the same general behavior is observable as with the corresponding DL-mixtures.<sup>4</sup> On the

TABLE I  
PAPAIN-CATALYZED SYNTHESIS OF PHENYLHYDRAZIDES OF ACYLATED D- AND L-PHENYLALANINES<sup>2</sup>

Acyl group	Configura- tion	M Substrate, concn.		Yield of phenylhydrazide, %			Total <sup>i</sup>
		Acid	Base	First fracn.	Second fracn.	Third fracn.	
CH <sub>3</sub> CO-	L	0.25	0.50	34 <sup>b</sup>	21 <sup>f</sup>	28 <sup>i</sup>	83
CH <sub>3</sub> CO-	D	.25	.50	...	...	...	0
C <sub>6</sub> H <sub>5</sub> CO-	L	.008	.016	82 <sup>b</sup>	21 <sup>f</sup>	...	103
C <sub>6</sub> H <sub>5</sub> CO-	D	.008	.016	4.5 <sup>c</sup>	4.5 <sup>g</sup>	...	9
C <sub>2</sub> H <sub>5</sub> OCO-	L	.025	.050	81 <sup>b</sup>	12 <sup>f</sup>	...	93
C <sub>2</sub> H <sub>5</sub> OCO-	D	.025	.050	61 <sup>d</sup>	15 <sup>h</sup>	...	76
C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> OCO-	L	.002	.004	32.5 <sup>b</sup>	24.5 <sup>f</sup>	12 <sup>i</sup>	70
C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> OCO-	D	.002	.004	30 <sup>b</sup>	2 <sup>f</sup>	...	32
C <sub>6</sub> H <sub>5</sub> SO <sub>2</sub> -	L	.004	.008	14 <sup>e</sup>	...	...	14
C <sub>6</sub> H <sub>5</sub> SO <sub>2</sub> -	D	.004	.008	...	...	...	0
C <sub>6</sub> H <sub>5</sub> NHCO-	L	.0025	.005	...	...	...	0
C <sub>6</sub> H <sub>5</sub> NHCO-	D	.0025	.005	...	...	...	0

<sup>a</sup> Enzyme concentration, 8 g. per liter; L-cysteine concentration 0.067 M; buffer concentration, 0.5 M acetic acid-0.5 M sodium acetate except for experiments with acetyl derivatives where concentration of the buffer was doubled; all reactions at 40° and pH 4.6 with 0.001 mole of acid except for the benzenesulfonyl- and N-phenylcarbonyl-compounds where the acid was 0.002 M. <sup>b</sup> After 10.5 hours. <sup>c</sup> After 221 hours. <sup>d</sup> After 117 hours. <sup>e</sup> After 20 days. <sup>f</sup> After additional 13 hours. <sup>g</sup> After additional 16 days. <sup>h</sup> After additional 19 days. <sup>i</sup> After additional 93 hours. <sup>j</sup> After the collection of the final fractions the pH of the filtrate was again determined; in no case was a substantial change from the initial pH of 4.6 observed.

whereas the other three, *i.e.*, carbomethoxy-, carboethoxy- and carbobenzoxy-, gave both D-

basis of information now at hand it appears reasonably certain that under the conditions specified cysteine activated papain is incapable of catalyzing the reaction between acetyl-D-phenylalanine and phenylhydrazine and that the reaction is restricted

(1) To whom inquiries regarding this article should be sent.

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

(3) E. I. Bennett and C. Niemann, *THIS JOURNAL*, **70**, 2610 (1948).

(4) E. I. Bennett and C. Niemann, *ibid.*, **72**, 1800 (1950).

(5) H. B. Milne and C. M. Stevens, *ibid.*, **72**, 1742 (1950).