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Hydrazides of *N*-Blocked Amino Acids as Sole Substrates with Unique Difunctional Behavior under Papain[1] Catalysis

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Hydrazides of five *N*-acylamino acids have been used alone as substrates for papain catalysis to yield N^1,N^2-diacylhydrazines. With the exception of *N*-(benzyloxycarbonyl)(Z)-D-alanine hydrazide, they were very effective as both acylating agents of the enzyme and nucleophiles in attacking the enzyme–substrate intermediate. Although Z-D-alanine hydrazide was a minimal acylating agent, it was a satisfactory nucleophile. The most favorable reaction involved Z-L-alanine hydrazide in producing N^1,N^2-bis(Z-L-alanyl)hydrazine. When Z-DL-alanine hydrazide was the substrate, this same chiral diacylhydrazine was formed along with meso N^1-(Z-D-alanyl)-N^2-(Z-L-alanyl)hydrazine. For the acylation step, the enzyme displayed powerful, essentially stereospecific, bias toward the L enantiomer. Once the thioester intermediate was formed, little preference was detected for attack by the enantiomers as nucleophiles. The most direct procedure for synthesis of substrates was conversion of Z-amino acids to their esters by means of dry HCl in an absolute alcohol. Treatment with hydrazine produced the hydrazides in excellent yield.

INTRODUCTION

Although hydrazides of *N*-blocked amino acids have never been introduced directly into reaction media as substrates for papain catalysis, twice before they have been implicated in an indirect manner, considerably before mechanistic details of papain catalysis were elucidated. In each instance, conditions were developed for successful reactions between an *N*-acylamino acid and hydrazine. Hippuric acid with a large excess of hydrazine responded to papain catalysis, yielding N^1,N^2-dihippurylhydrazine (*1*). In a similar situation, Z-DL-alanine[2] and hydrazine gave rise to N^1,N^2-bis(Z-L-alanyl)hydrazine (*2*). On the basis of contemporary information concerning the structure of papain and its catalysis (*3*), it can be surmised that Z-L-alanine first acylates the mercapto group of the enzyme, with subsequent nucleophilic attack by hydrazine on the thioester to form soluble Z-L-alanine hydrazide:

$$\text{Z-DL-Ala-OH} + \text{Enz-S-H} \rightarrow \begin{matrix}\text{Z-L-Ala}\\ |\\ \text{Enz-S}\end{matrix} + \text{H}_2\text{O} \xrightarrow{\text{NH}_2\text{NH}_2} \text{Z-L-Ala-NHNH}_2 + \text{Enz-S-H}.$$

[1] Very active crude material readily isolated from papaya latex.

[2] Z is the accepted abbreviation for *N*-(benzyloxycarbonyl).

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As soon as sufficient hydrazide is formed, it effectively competes with hydrazine as a nucleophile in attacking the thioester intermediate, thereby producing the N^1,N^2-diacylhydrazine:

$$\begin{matrix}\text{Z-L-Ala}\\ |\\ \text{Enz-S}\end{matrix} \xrightarrow{\text{NH}_2\text{-NH-L-Ala-Z}} \begin{matrix}\text{Z-L-Ala-NH}\\ |\\ \text{Z-L-Ala-NH}\end{matrix} + \text{Enz-S-H.}$$

A balance would be established between continuous formation of the hydrazide and its concurrent removal in production of insoluble N^1,N^2-diacylhydrazine. The hydrazide is evidently a more effective nucleophile than hydrazine, because in one instance the ratio of hydrazine to racemic Z-DL-alanine was very large (*2*). Since papain is essentially stereospecific in favoring Z-L-alanine during the acylation step, the resultant hydrazide and N^1,N^2-diacylhydrazine must be chiefly L in configurational content.

In the research reported here, the hydrazides selected for utilization as substrates were hippuric, Z-glycine, Z-L-alanine, Z-D-alanine, and Z-DL-alanine hydrazides. The investigation consisted of five phases. First, even though all of these hydrazides were known, better procedures were sought for their synthesis than formerly described. Second, achiral hippuric hydrazide and Z-glycine hydrazide actually did form insoluble N^1,N^2-dihippurylhydrazine and N^1,N^2-bis(Z-glycyl)hydrazine, respectively. This finding supported the previous conjecture that soluble hydrazides were formed when combinations of *N*-acylamino acids with hydrazine were the substrates:

$$\text{R-CO-NHNH}_2 + \text{Enz-S-H} \rightarrow \begin{matrix}\text{R-CO}\\ |\\ \text{Enz-S}\end{matrix} + \text{NH}_2\text{NH}_2 \xrightarrow{\text{NH}_2\text{NH-CO-R}} \begin{matrix}\text{R-CO-NH}\\ |\\ \text{R-CO-NH}\end{matrix} + \text{Enz-S-H.}$$

Third, Z-L-alanine hydrazide by itself readily formed pure Z-L-Ala-NH-NH-L-Ala-Z, whose properties have been described (*2*). Fourth, Z-D-alanine hydrazide was a very ineffective substrate for producing the N^1,N^2-diacylhydrazine. Fifth, the most novel portion of the research emerged when racemic Z-DL-alanine hydrazide was the substrate: the insoluble product was a diastereoisomeric mixture of N^1,N^2-diacylhydrazines.

RESULTS AND DISCUSSION

Synthesis of Hydrazides of N-Acylamino Acids

Hippuric hydrazide was previously synthesized (*4*, *5*) by starting with hippuric acid, then converting it into its ethyl ester by means of ethanol and H_2SO_4, followed by treatment with 64% aqueous hydrazine in ethanol. In the present research an alternate method for the preparation of ethyl hippurate involved an adaptation of the use of a catalytic dehydrator that had been utilized in synthesizing

ethyl acetate and other simple esters (*6*). The catalytic dehydrator, Dowex H-Form resin plus anhydrous $CaSO_4$, produced methyl hippurate from absolute methanol and hippuric acid. Methyl hippurate was then subjected to 98.5% aqueous hydrazine in methanol to yield the hydrazide (Table 1). Z-Glycine hydrazide was prepared in a similar manner.

The method of synthesis reported (*7*) for Z-D-alanine hydrazide and Z-L-alanine hydrazide started with the individual ethyl D- or L-alaninate hydrochloride, which was converted into ethyl Z-D- or Z-L-alaninate. Toward this end, benzyl chloroformate was used to acylate the free amino group of ethyl D- or L-alaninate. This reaction required careful release of this group by a base in such a manner that HCl from the ester hydrochloride was removed. In addition, HCl produced by acylation of the amino group with benzyl chloroformate, would also be removed. Chloroform separated ethyl Z-D- and Z-L-alaninate from the aqueous mixture. These isolated ethyl esters were treated with aqueous 64% hydrazine, thus yielding the desired hydrazides. Z-Glycine hydrazide has also been prepared by this same procedure.

However, a simpler approach for the preparation of Z-glycine hydrazide has also been utilized (*8*). In the current research, this method proved not only to be the most effective for this hydrazide, but also was readily applied as a new approach to Z-D-, Z-L-, and Z-DL-alanine hydrazides (Table 1). For example, Z-glycine in a dry methanolic solution of hydrogen chloride produced methyl Z-glycinate. Isolation of this ester and subjection to a reaction with an ethanolic solution of 98.5% aqueous hydrazine gave the desired hydrazide. The only reported synthesis of Z-DL-alanine hydrazide was contained in a patent (*9*). The preparation involved the use of Z-DL-alanine, an H-Form resin and epichlorohydrin, thus forming an ester of Z-DL-alanine. When exposed to hydrazine, Z-DL-alanine hydrazide was obtained.

It should be noted that two of these N^1,N^2-diacylhydrazines have been previously prepared by nonenzymic procedures. N^1,N^2-Dihippurylhydrazine was formed as a by-product during the preparation of hippuric hydrazide from ethyl

TABLE 1

SYNTHESES OF SUBSTRATE HYDRAZIDES

Hydrazide	mp (°C)	$[\alpha]_D^{25}$ in 0.50 *M* HCl	Percentage yield	Percentage N	
				Calcd	Found
Hippuric[a]	163–165	Achiral	45	21.75	21.44
Z-Glycine[a]	108–110	Achiral	40	18.83	18.85
Z-Glycine[b]	108–110	Achiral	87	18.83	18.85
Z-D-Alanine[b]	135–136	+28.6°	86	17.71	17.70
Z-L-Alanine[b]	135–136	−28.8°	84	17.71	17.40
Z-DL-Alanine[b]	116–117	Racemic	80	17.71	17.35

[a] From catalytic dehydrator method.

[b] From dry HCl–alcohol method.

hippurate and hydrazine (*4*). It has also been synthesized by a deliberate reaction between hippuric hydrazide and ethyl hippurate as well as cyanomethyl hippurate (*10*). N^1,N^2-Bis(Z-glycyl)hydrazine was reported (*8*) as a by-product during the synthesis of a polypeptide which demanded the intermediate preparation of Z-glycyl-L-leucine methyl ester. During this ester synthesis, Z-glycyl azide was prepared from Z-glycine hydrazide. Formation of the azide from the hydrazide was incomplete. Therefore, an inadvertant reaction between the azide and the residual hydrazide occurred, thus yielding the diacylhydrazine.

Acylation of Papain and Nucleophilic Attack on the Resultant Thioester

When *N*-acylamino acids have been utilized for acylation of papain, many types of compounds that contained an appropriate amino functional group qualified for effecting a nucleophilic attack on the resultant thioester intermediate. Among these have been aniline (*11*), benzyhydrazide (*12*), benzenesulfonhydrazide (*13*), phenylhydrazine and substituted phenylhydrazines (*14*), hydrazides that incorporated a heterocyclic nucleus (*15*), and also anilides of amino acids (*16*). Of course, the hydrazides mentioned could not acylate the enzyme, but acylation was achieved by amides of *N*-acylamino acids as well as *N*-acylamino acids themselves. Hippuric amide (*17, 18*) and Z-L-alanine amide (*18*) performed this function with ease, as the necessary step before an attack by aniline to yield hippuric anilide and Z-L-alanine anilide. The well-known amide, *N*-benzoyl-L-arginine amide, is a similar acylating agent, whose hydrolysis has been exploited for determination of the activity (*19*) of papain preparations.

The rationale for using special hydrazides as sole substrates was provided when reactions of the amides, as well as the behavior of *N*-acylamino acids toward hydrazine, were related to more recent development of mechanistic details (*3*) of papain catalysis. With appropriate configurational content, the carbonyl (I) of the hydrazide functional group should acylate the enzyme, with concurrent release of

TABLE 2

N^1,N^2-DIACYLHYDRAZINES FROM CHIRAL OR ACHIRAL HYDRAZIDE SUBSTRATES

N^1,N^2-Diacylhydrazine	mp (°C)	$[\alpha]_D^{25}$ in DMF	Percentage yield	Percentage N	
				Calcd	Found
Hipp-N-H | Hipp-N-H					
Z-Gly-N-H | Z-Gly-N-H	252–254	Achiral	54	15.81	15.96
Z-L-Ala-N-H | Z-L-Ala-N-H	218–219	Achiral	68	13.52	13.77
Z-D-Ala-N-H | Z-D-Ala-N-H	246–247	−21.7	72	12.66	12.84
Z-D-Ala-N-H | Z-L-Ala-N-H	244–246	—	3	12.66	12.48

TABLE 3

PAPAIN-CATALYZED REACTIONS OF Z-DL-ALANINE HYDRAZIDE ALONE TO FORM MIXTURES[a] OF Z-L-ALA-NH-NH-L-ALA-Z WITH MESO Z-D-ALA-NH-NH-L-ALA-Z

Incubation period (hr)	mp (°C)	$[\alpha]_D^{25}$ in DMF	Percentage meso in mixture	Percentage N	
				Calcd	Found
1–2	232–234	−9.20°	58	12.66	12.87
2–3	245–247	−4.20°	81	12.66	12.43
3–4	246–247	−3.10°	86	12.66	12.45
4–5	248–251	−2.30°	89	12.66	12.33
5–6	249–251	−1.30°	94	12.66	12.33
6–7	249–251	−0.40°	98	12.66	12.48
24–48	253–255	0.00	100	12.66	12.94

[a] Total yield 68%, which did not include 0- to 1-hr induction period.

hydrazine. The amino portion (II) of a second molecule could then serve as a

$$\underset{\text{I}}{\text{RCO-NH-CHR'-CO-NH-NH}_2}$$

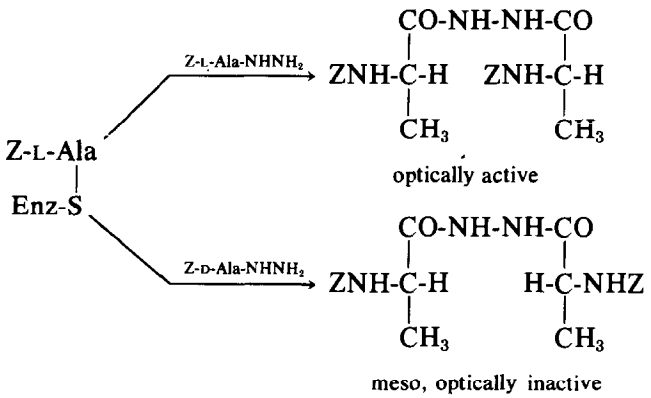
$$\text{II}$$

nucleophile in yielding an N^1,N^2-diacylhydrazine. The few selected hydrazides were exploited to reveal unique factors of stereochemical control that papain could foster when these substrates were single reactants, without the presence of a different type of nucleophile. Some of the important features of the study are summarized in Tables 2 and 3.

The trial usage of achiral hippuric hydrazide and Z-glycine hydrazide conclusively demonstrated that these kinds of hydrazides behave as excellent acylating agents of the enzyme and subsequently as nucleophiles to produce the anticipated N^1,N^2-diacylhydrazines in good yield. Z-L-Alanine hydrazide was highly productive in rapidly yielding optically pure N^1,N^2-bis-(Z-L-alanyl)hydrazine. The specific rotation in dimethylformamide (DMF) was slightly higher than for the product formed from Z-DL-alanine and hydrazine (*2*). On the other hand, Z-D-alanine hydrazide yielded an almost negligible amount of the diacylhydrazine over an extended incubation period of several days, demonstrating papain's preference for the L enantiomer, bordering on stereospecificity.

On the basis of the extreme stereoselectivity shown by papain in favoring Z-L-alanine hydrazide rather than the D enantiomer for acylation, a clear interpretation is afforded for the results obtained from Z-DL-alanine hydrazide as the substrate. It can be assumed that acylation of the enzyme was attributable almost entirely to the L enantiomer of the racemic modification, particularly because the D enantiomer now had to compete for a catalytic site with a highly favored L competitor, thereby decreasing still further the meager ability of the D enantiomer to form the thioester. However, both enantiomers of the racemic substrate were able to exert nucleophilic behavior. No excessive preference was displayed by the

enzyme for the hydrazide during the formation of the diastereoisomeric mixture of N^1-(Z-D-alanyl)-N^2-(Z-L-alanyl)hydrazine and N^1,N^2-bis(Z-L-alanyl)hydrazine:



Depletion of the L hydrazide was more rapid than that of the D enantiomer, due to participation of the L hydrazide as an acylating agent and a nucleophile, with particular enzymic bias toward the L hydrazide at the time of acylation. In Table 3, some of the pertinent details are provided. The specific rotation of the solid product mixture dropped continuously for the progressive incubation periods. After a few hours, the product was essentially only the meso compound with a zero optical rotation. Lower melting points were shown at the onset and progressed to a maximum at about 253–255°C, in agreement with Z-D-Ala-NH-NH-L-Ala-Z prepared from Z-L-alanine and Z-D-alanine hydrazide.

EXPERIMENTAL

Isolation and Activation of Crude Papain

This procedure involves significant modifications of the method used by Bennett and Niemann (*20*). Papaya latex (100 g) was stirred 4 hr with 450 ml of ice-cold water in an ice bath. After cold centrifugation for 30 min at 10,000 rpm, the supernatent liquid was suction filtered. H_2S was passed through the solution for 18 hr, maintained in an ice bath. A small amount of Hyflo Super-Cel filter aid was added with shaking, followed by cold centrifugation for 30 min at 10,000 rpm. The solution was filtered twice followed by addition of dry MeOH to produce 70% by volume of MeOH. The paste was removed by centrifugation at 10,000 rpm for 30 min and then dried under a vacuum over P_2O_5 to yield 24 g of brittle, very active papain. It was stored in a refrigerator in a screw-cap bottle sealed with masking tape.

Activity of Crude Papain

About 10 g of the crude papain was ground to homogeneous powder in an agate mortar, carefully sealed, and stored separately under refrigeration for the current

research. Activity was tested on three samples for catalysis of the reaction between Z-glycine and aniline, using a modification of the procedure described by Bergmann and Frankel-Conrat (*17*) for the synthesis of Z-glycine anilide. Quantities of materials were: 0.250 g papain, 0.250 g L-cysteine · HCl · H_2O, 0.0100 mol Z-glycine, and 0.0100 mol aniline in 100 ml 0.5 *M* acetate buffer, pH 4.5. The yield of product after a 24-hr incubation period at 40°C was higher than for any other crude papain preparation in this laboratory and was reproducible in the three independent trials.

Synthesis of Hydrazides via the Catalytic Dehydrator Method

Hippuric hydrazide. A mixture of dried hippuric acid (10 g), dried Dowex 50W-X8 H-Form ion-exchange resin (10 g), and dried Drierite, anhydrous $CaSO_4$ (23.3 g), was stirred with 125 ml of anhydrous MeOH for 1.5 hr at room temperature. After standing for 5 days at room temperature, the resin and calcium salts were removed by suction filtration and washed with a small quantity of MeOH. The combined filtrates were filtered twice more, first through a Büchner funnel, then through a sintered glass funnel. Solution was then evaporated on a rotary evaporator to yield methyl hippurate as a yellow, viscous oil, which was dissolved in anhydrous MeOH (20 ml) and added dropwise to a hydrazine (2.6 ml, 98.5%) and MeOH (50 ml) solution in an ice bath over a period of 1.5 hr. The small amount of precipitate that formed during addition was removed by filtration and the clear filtrate was stored overnight in a refrigerator. The precipitate that formed was removed by suction filtration. Most of the solid dissolved in hot EtOH and the solution was filtered through a hot water-jacketed Büchner funnel. On being cooled, white, crystalline solid formed from the filtrate and was removed by suction filtration. Dried product weighed 5.2 g. For mp, percentage N, and percentage yield, see Table 1.

Z-Glycine hydrazide. Quantities of materials and experimental procedure were identical to those for preparation of hippuric hydrazide except Z-glycine (10 g) replaced hippuric acid. Melting point, percentage N, and percentage yield for the product (4.3 g) are listed in Table 1.

Synthesis of Hydrazides via a Dry HCl–Alcohol Method (*8*)

Z-DL-Alanine hydrazide. Technical grade HCl gas (3.65 g) was dissolved in 100 ml of anhydrous MeOH in a 250-ml round-bottom flask immersed in an ice bath under the hood. Z-DL-Alanine (10 g) was added in small portions, until completely dissolved, then sealed and allowed to stand at room temperature for 36 hr. Two pieces of bent glass rod were added to prevent bumping while the MeOH and HCl were removed on a rotary evaporator at 30°C. An aspirator was used to maintain reduced pressure for 2.5 hr. The reaction flask was sealed and stored in the refrigerator for 24 hr. The solidified methyl Z-DL-alaninate was dissolved in 35 ml of anhydrous MeOH and transferred to a small dropping funnel. The methyl ester solution was then added dropwise to a hydrazine (2 g, 98.5%) and anhydrous MeOH (15 ml) solution over a period of 30 min. The evaporator flask and funnel were rinsed with anhydrous MeOH (10 ml), which was added dropwise to the

hydrazine solution. The resultant solution was transferred to a round-bottom flask, sealed, and allowed to stand for 24 hr at room temperature. Then MeOH was removed on a rotary evaporator at 30°C. The crystals of Z-DL-alanine hydrazide were dissolved in hot ethyl acetate and filtered through a hot water-jacketed Büchner funnel. On cooling, white crystalline solid was collected on an ice-cold water-jacketed Büchner funnel and dried (8.5 g) over P_2O_5. Mp, percentage N, and percentage yield are given in Table 1.

Z-D-Alanine hydrazide. Experimental details were identical with procedure outlined for synthesis of Z-DL-alanine hydrazide but Z-D-alanine (10 g) was substituted for Z-DL-alanine. Crude Z-D-alanine hydrazide (9.1 g) was recrystallized from ethyl acetate. α_{obs} in 0.50 *M* HCl + 0.573°, c = 0.02000 g/ml, 1-dm tube at 25°C, D-line sodium. For $[\alpha]_D^{25}$, mp, percentage N, and percentage yield, see Table 1.

Z-L-Alanine hydrazide. Z-L-Alanine (10 g) was used to replace Z-DL-alanine in the same experimental procedure as described for the preparation of Z-DL-alanine hydrazide. Crude Z-L-alanine hydrazide (8.9 g) was recrystallized from ethyl acetate. α_{obs} in 0.50 *M* HCl − 0.575°, c = 0.02000 g/ml, 1-dm tube, 25°C, D-line sodium. $[\alpha]_D^{25}$, mp, percentage N, and percentage yield are listed in Table 1.

Z-Glycine hydrazide. Z-Glycine (10 g) was used under otherwise identical conditions as in the preceeding synthesis. Crude Z-glycine hydrazide (8.7 g) was recrystallized from ethyl acetate. For mp, percentage N, and percentage yield, see Table 1.

Synthesis of N^1,N^2-Diacylhydrazines from Hydrazides of N-Acylamino Acids under Papain Catalysis

N^1,N^2-Dihippurylhydrazine from hippuric hydrazide. Active papain (0.4000 g) and an equal weight of L-cysteine · HCl · H_2O were combined and pulverized in a porcelain mortar with a pestle, then dissolved in 30 ml of acetate buffer (0.5 *M*, pH 4.5). Hippuric hydrazide (2.000 g) was similarly ground and dissolved completely in 170 ml of acetate buffer (0.5 *M*, pH 4.5). Both solutions were filtered through sintered glass funnels, mixed in a glass-stoppered flask, and allowed to react in an incubator at 40°C. The resultant precipitates were removed by suction filtration at successive time intervals and rinsed thoroughly with deionized water. Products were dried over P_2O_5 in a vacuum desiccator and weighed: 0–2 hr, 0.2961 g; 2–4 hr, 0.1788 g; 4–24 hr, 0.4501 g; 24–48 hr, 0.0498 g. Mp, percentage N, and percentage yield are listed in Table 2.

N^1,N^2-Bis(Z-glycyl)hydrazine from Z-glycine hydrazide. Z-Glycine hydrazide (0.5000 g) was dissolved in 40 ml of acetate buffer (0.5 *M*, pH 4.5) and the powdered mixture of active papain (0.1000 g) and L-cysteine · HCl · H_2O (0.1000 g) was dissolved in 10 ml of acetate buffer (0.5 *M*, pH 4.5). Solutions were subsequently filtered through sintered glass funnels, combined in a glass-stoppered flask and incubated at 40°C. At appropriate intervals, product was removed by suction filtration, washed with deionized water during applied suction, and then dried over P_2O_5 under vacuum. Product: 0–4 hr, 0.1094 g; 4–18 hr, 0.2008 g; 18–48 hr, 0.0050 g. For mp, percentage N, and percentage yield, see Table 2.

N^1,N^2-Bis(Z-L-alanyl)hydrazine from Z-L-alanine hydrazide. Active papain (0.2000 g) and L-cysteine · HCl · H_2O (0.2000 g) were dissolved in 30 ml of acetate buffer (0.5 *M*, pH 4.5). The solution was filtered through a sintered glass funnel. Z-L-Alanine hydrazide (0.9703 g) was dissolved completely in 70 ml of acetate buffer (0.5 *M*, pH 4.5), filtered through a sintered glass funnel, combined with the papain solution, and incubated at 40°C. Product was collected at timed intervals, washed thoroughly with deionized water, and dried over P_2O_5: 0–4 hr, 0.5119 g; 4–12 hr, 0.1031 g; 12–36 hr, 0.0317 g. α_{obs} in DMF – 0.434° (0–4 hr), c = 0.02000 g/ml, 1-dm tube at 25°C, D-line sodium. $[\alpha]_D^{25}$, mp, percentage N, and percentage yield are listed in Table 2.

N^1,N^2-Bis(Z-D-alanyl)hydrazine from Z-D-alanine hydrazide. Experimental procedure was identical with synthesis of N^1,N^2-bis(Z-L-alanyl)hydrazine except Z-D-alanine hydrazide (0.9967 g) was substituted for Z-L-alanine hydrazide. Product was collected over extended time periods: 0–22 hr, 0.0186 g; 22–51 hr, 0.0099 g. Measurement of optical rotation was not possible due to insufficient precipitate. Melting point, percentage N, percentage yield are listed in Table 2.

N^1,N^2-Bis(Z-L-alanyl)hydrazine and N^1-(Z-D-Alanyl)-N^2-(Z-L-alanyl)hydrazine from Z-DL-alanine hydrazide. Active papain (1.011 g) and L-cysteine · HCl · H_2O (1.008 g) were pulverized in a mortar, dissolved in 50 ml of acetate buffer (0.6 *M*, pH 4.5), and filtered through a sintered glass funnel. Z-DL-Alanine hydrazide (4.8735 g) was similarly dissolved in 450 ml of acetate buffer (0.6 *M*, pH 4.5), and filtered through a sintered glass funnel. Both solutions were combined in a glass-stoppered flask and incubated at 40°C. After short periods of incubation, the product was removed by suction filtration, washed with deionized water, and dried over P_2O_5 in a vacuum desiccator. Optical rotations were determined at 25° in DMF, D-line sodium, c = 0.01000 g/ml, 1-dm tube. Incubation period, weight product, α_{obs}: 1–2 hr, 0.3818 g, α_{obs} – 0.092°; 2–3 hr, 0.3278 g, α_{obs} – 0.042°; 3–4 hr, 0.2762 g, α_{obs} – 0.031°; 4–5 hr, 0.2003 g, α_{obs} – 0.023°; 5–6 hr, 0.1912 g, α_{obs} – 0.013°; 6–7 hr, 0.1710 g, α_{obs} – 0.004°; 7–8 hr, 0.1462 g; 8–9 hr, 0.1355 g; 9–10 hr, 0.1218 g; 10–11 hr, 0.1090 g; 11–12 hr, 0.0847 g; 12–18 hr, 0.2857 g; 18–24 hr, 0.2315 g; 24–48 hr, 0.3393 g, α_{obs} 0.000°; 48–72 hr, 0.1116 g; 72–96 hr, 0.0560 g; 96–120 hr, 0.0097 g; 120–192 hr, 0.0027 g. For $[\alpha]_D^{25}$, mp, percentage N, and percentage yield, see Table 3.

Measurement of Optical Rotations

High observed rotations were measured in a Rudolph Model 80 high-precision polarimeter and measurements of low rotations in a Perkin–Elmer Model 243 polarimeter.

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REFERENCES

1. C. NIEMANN AND P. L. NICHOLS, JR., *J. Biol. Chem.* **143,** 19 (1942).
2. F. W. HOLLY, J. J. CAHILL, JR., AND K. FOLKERS, *J. Amer. Chem. Soc.* **73,** 2944 (1951).
3. A discussion on the structures and functions of proteolytic enzymes. *Phil. Trans. Roy. Soc. London, Ser.* **B 257,** 66–266 (1970).
4. T. CURTIUS, *Berichte* **23,** 3023 (1890).
5. T. CURTIUS, *J. Prakt. Chem.* **52,** 11 (1895).
6. G. F. VESLEY AND V. I. STENBERG, *J. Org. Chem.* **36,** 2548 (1971).
7. B. F. ERLANGER AND E. BRAND, *J. Amer. Chem. Soc.* **73,** 3508 (1951).
8. S. SIMONDS, J. I. HARRIS, AND J. S. FRUTON, *J. Biol. Chem.* **188,** 259 (1951).
9. H. JESCHKEIT, *Chem. Abstr.* **70,** 393 (1969) (East German patent).
10. S. GRUDZINSKI, *Rocz. Chem.* **33,** 655 (1959) from *Chem. Abstr.* **54,** 2148 (1960).
11. D. G. DOHERTY AND E. A. POPENOE, JR., *J. Biol. Chem.* **189,** 447 (1951).
12. J. L. ABERNETHY, M. KIENTZ, R. JOHNSON, AND R. JOHNSON, *J. Amer. Chem. Soc.* **81,** 3944 (1959).
13. J. L. ABERNETHY, L. YENGOYAN, J. SEAY, AND J. ABU-SAMRA, J. *Org. Chem.* **27,** 2528 (1962).
14. J. L. ABERNETHY, E. ALBANO, AND J. COMYNS, *J. Org. Chem.* **189,** 1580 (1971).
15. J. L. ABERNETHY, D. SRULEVITCH, AND M. J. ORDWAY, JR., *J. Org. Chem.* **40,** 3445 (1975).
16. S. W. FOX, M. WINITZ, AND C. W. PETTINGA, *J. Amer. Chem. Soc.* **75,** 5539 (1953).
17. BERGMANN AND H. FRAENKEL-CONRAT, *J. Biol. Chem.* **119,** 707 (1937).
18. J. L. ABERNETHY, F. G. HOWELL, A. LEDESMA, D. DOOSE, AND R. EVERETT, *Tetrahedron* **31,** 2693 (1975).
19. J. R. KIMMEL AND E. L. SMITH, *Biochem. Prep.* **6,** 61 (1958).
20. E. L. BENNETT AND C. NIEMANN, *J. Amer. Chem. Soc.* **72,** 1798 (1950).