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Stereoselective Action of Acylated Crude Papain[1] toward Mandelic and Atrolactic Hydrazides

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Acylated crude papain has been shown to exert stereoselective behavior toward racemic hydrazides devoid of an amino acid residue, namely, (*RS*)-mandelic and (*RS*)-atrolactic hydrazides. These hydrazides functioned as nucleophiles to yield N^1,N^2-diacylhydrazines. Several achiral acylating agents for the enzyme were chosen, including *Z*-glycine, BOC-glycine, AOC-glycine, and hippuric acid. With the exception of hippuric acid as the acylating agent, the reaction product, in every instance for these achiral hydrazides, consisted of an excess of the (+)-N^1,N^2-diacylhydrazine. The relative rates of product formation for the mandelic hydrazides were considerably greater than for corresponding reactions with racemic atrolactic hydrazide. When chiral *Z*-L-alanine was employed to acylate crude papain, the stereoselective action was most pronounced, with the formation of a mixture of diastereoisomers consisting of 73% N^1-(*Z*-L-alanyl)-N^2-[(*R*)-mandelyl]hydrazine. The relative reactivities for the electrophiles was *Z*-L-alanine >>> *Z*-glycine ≃ hippuric acid >> AOC-glycine > BOC-glycine. The hydrazides of (*R*)-, (*S*)-mandelic, and (*RS*)-atrolactic acids were prepared by conversion of the corresponding acids to their esters by means of a catalytic dehydrating agent and subsequent treatment with a methanolic solution of hydrazine.

INTRODUCTION

In previous research, hydrazides of *N*-acylamino acids have been used as sole substrates for catalysis by crude papain (*1*). Subsequently, they served as electrophiles when excess aniline was the nucleophile or as nucleophiles when an excess of an *N*-acylamino acid was the electrophile (*2*). As an electrophile, racemic *Z*-DL-alanine hydrazide[2] displayed an essentially stereospecific response in acylating the enzyme. The subsequent attack by aniline yielded *Z*-L-alanine anilide. When this same racemic hydrazide was forced, by the use of an *N*-blocked amino acid, to behave as a nucleophile, a stereoselective response was exhibited.

The continuing firm interest in facets of stereoselectivity (*3–5*) led us to explore whether or not incorporation of an amino acid residue within the structure of a racemic hydrazide was necessary in order to obtain such a stereoselective response toward acylated crude papain. The hydrazides selected for this investiga-

[1] This preparation of crude papain contains a mixture of sulfhydryl proteolytic enzymes.

[2] Abbreviations used: *Z*, *N*-(benzyloxycarbonyl); AOC- and BOC-, *N*-(tert-amyloxycarbonyl) and *N*-(tert-butyloxybarbonyl).

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tion were mandelic and atrolactic hydrazides. These particular hydrazides were especially favorable because they are reasonably soluble in an aqueous medium due to their alcoholic hydroxyl groups. Also, the corresponding acids have played a significant role in the progressive development of stereoorganic chemistry, extending over a period of several decades (*6–14*). Synthetic procedures for mandelic and atrolactic acids are well known (*15–17*). Resolutions of the racemic mandelic and atrolactic acids have involved the use of alkaloids (*6, 10, 11, 18–20*), *R* and *S* phenethyl amine (*11, 21*) and L-asparagine (*22*). The absolute configurations of these resolved acids have been established by a variety of means (*10, 23–25*).

Research Objectives

The principal objectives of this research were (a) synthesis of the necessary hydrazides; (b) use of hippuric acid, *Z*-glycine, *Z*-L-alanine, AOC-glycine, and BOC-glycine to acylate the enzyme; (c) the use of appropriate mandelic and atrolactic hydrazides in testing for stereoselectivity; (d) special observation with *Z*-L-alanine for possible disclosure of increased stereoselectivity due to its chiral nature; and (e) use of commercial chymopapain (Sigma Chemical Co., St. Louis, Mo.), free from papain, to reveal its actual catalytic involvement and relative stereoselective behavior.

RESULTS AND DISCUSSION

Synthesis of Hydrazides (See Table 1)

(*RS*)-Mandelic hydrazide was readily prepared from commercial methyl (*RS*)-mandelate by treatment with a methanolic solution of hydrazine. The two chiral mandelic acids were converted to their methyl esters by a procedure that used a catalytic dehydrating agent (*1, 2, 26*). The esters were then exposed to a methanolic solution of hydrazine to yield the desired hydrazides. In the case of methyl (*RS*)-atrolactate, racemic atrolactic acid hemihydrate was first dried over phosphorus pentoxide in a vacuum dessicator and then treated similarly with hydrazine.

TABLE 1

SYNTHESIS OF SUBSTRATE HYDRAZIDES[a]

Product	Melting point (°C)	$[\alpha]_D^{25}$ in pyridine (°)	Yield (%)	%N	
				Calcd	Found
(*RS*)-Mandelic hydrazide	132–133	0.0	74.1	16.91	16.60
(*R*)-Mandelic hydrazide	144–146	−37.4	52.6	16.91	16.90
(*S*)-Mandelic hydrazide	144–146	+38.6	54.1	16.91	17.10
(*RS*)-Atrolactic hydrazide	110–112	0.0	29.6	15.55	15.79

[a] See Experimental for reaction conditions.

Stereoselective Action Exerted by Acylated Crude Papain toward the Hydrazides of Mandelic and Atrolactic Acids

Although it might be assumed that hydrazides lacking an amino acid residue would not acylate the crude enzyme and then produce an insoluble diacylhydrazine (*27, 28*), it was necessary to determine if indeed this was the case with mandelic and atrolactic hydrazides. When racemic mandelic and atrolactic hydrazides were tested as potential sole substrates, in the presence of crude papain, no reaction was evident. The hydrazides were found to react as nucleophiles in every case where various *N*-acylamino acids were used first to acylate the crude enzyme:

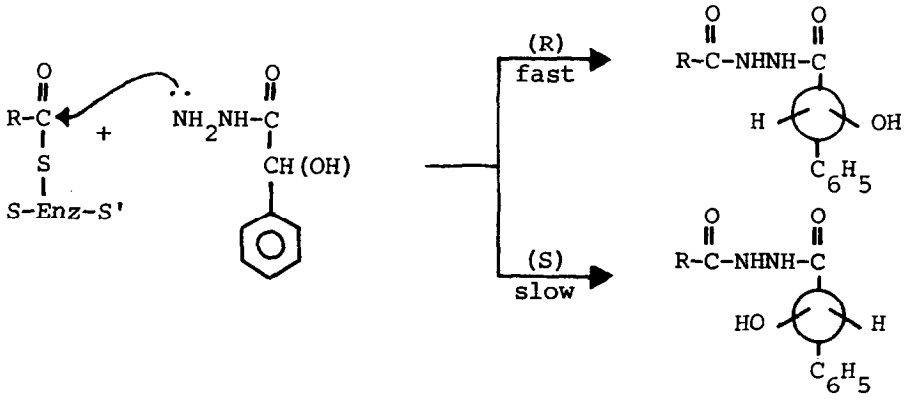
$$\text{Enz—SH} \xrightarrow[\text{electrophile}]{\text{RCO—OH}} \text{Enz—S—COR} \xrightarrow[\text{nucleophile}]{\text{NH}_2\text{NH—COR}'} \begin{matrix} \text{R'CO—NH} \\ | \\ \text{RCO—NH.} \\ + \\ \text{Enz—SH} \end{matrix} \qquad [1]$$

$$\text{Enz—S—COR} + \text{H}_2\text{O}$$

RCO represents an appropriate *N*-blocked amino acid residue, whereas R'CO represents a mandelyl or atrolactyl group.

Unlike the hydrazides of certain *N*-acylamino acids (*1, 2*), mandelic and atrolactic hydrazides were not able to acylate the crude enzyme. This behavior could be the result of an inability to induce the proper enzyme conformation change at the S-subsite region (*29, 30*); or possibly these hydrazides can fit with proper orientation for acylation, but lack sufficient binding interactions to permit the formation of the thioester. Mandelic and atrolactic hydrazides, however, were found to be effective reactants at the S'-subsite region of the active site (*29, 30*), as evidenced by their nucleophilic action in attacking the acylated crude enzyme.

It became evident that the nature of the substrate responsible for acylation determines the extent of subsequent stereoselective action exerted toward the racemic hydrazides. In every case where stereoselective action was observed, preference was given to the *R* hydrazide:



[2]

The greatest stereoselective response, involving the crude enzyme acylated with an achiral electrophile, with either racemic hydrazide, was observed for

TABLE 2

COMPARATIVE DATA FOR N^1,N^2-DIACYLHYDRAZINES PRODUCED IN REACTIONS OF ACYLATED CRUDE PAPAIN WITH (*RS*)-MANDELIC HYDRAZIDES[a]

Acylating agent	Principal product	Yield (%)	*R* in first incubation period (%)
Z-Gly-OH	*Z*-Gly-NH (*R*)-Mand-NH	40.1	63.7
Hipp-OH	Hipp-NH (*RS*)-Mand-NH	44.2	50.0
AOC-Gly-OH	AOC-Gly-NH (*R*)-Mand-NH	29.4	67.5
BOC-Gly-OH	BOC-Gly-NH (*R*)-Mand-NH	28.5	59.9

[a] See Experimental for reaction conditions.

AOC-glycine (Table 2). Moderate stereoselectivity was also realized with BOC-glycine and *Z*-glycine (Table 2). When hippuric acid was the acylating agent, stereoselectivity was not shown toward (*RS*)-mandelic hydrazide, whereas when (*RS*)-atrolactic hydrazide was the nucleophile, an optically active product was obtained.

Utilization of optically active *Z*-L-alanine to acylate the enzyme gave rise to enhanced stereoselectivity when racemic mandelic hydrazide was the nucleophile. The first incubation period produced 73% N^1-(*Z*-L-alanyl)-N^2-[(*R*)-mandelyl]hydrazine (Figs. 1 and 2; see Experimental, *Z*-L-Alanine and (*RS*)-mandelic hydrazide). *Z*-L-Alanine was expressly used for the formation of a mixture of

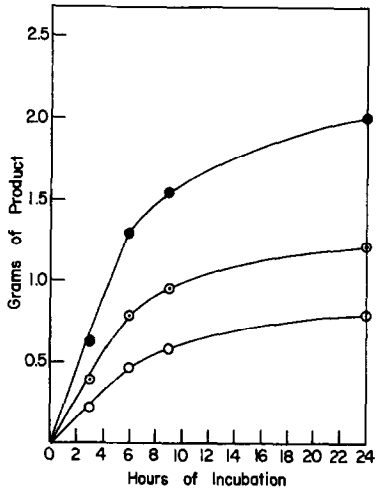


FIG. 1. Rates of precipitation of N^1-(*Z*-glycyl)-N^2-[mandelyl]hydrazines for reaction between *Z*-glycine and (*RS*)-mandelic hydrazides under catalysis by crude papain at 40°C. ●, mixture of enantiomers; ⊙, (*R*)-product in mixture; ○, (*S*)-product in mixture.

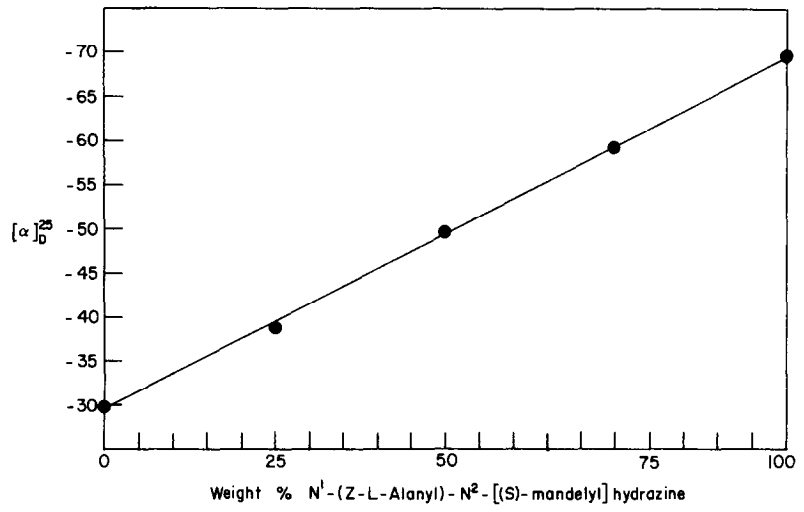


FIG. 2. Specific rotation vs percent composition for mixtures of N^1-(*Z*-L-alanyl)-N^2-[(*R*)-mandelyl]hydrazine and N^1-(*Z*-L-alanyl)-N^2-[(*S*)-mandelyl]hydrazine.

diastereoisomers from racemic mandelic hydrazide, because there might be an enhanced difference in rates of formation of isomeric products. In order to assess the extent of stereoselective action, it was necessary to prepare a standard curve (Fig. 2) that displayed optical rotations and corresponding percent composition for known mixtures of the two diastereoisomers (Table 3).

This research revealed that acylated crude papain can exert stereoselective influence toward a nucleophilic reactant that lacks an amino acid residue.

Preliminary research on fractionation of crude papain by ion exchange chromatography has shown two active protein fractions which may be identified as chymopapain and papain. Each peak displayed a preference for the *R* hydrazide in the reaction between *Z*-L-alanine and (*RS*)-mandelic hydrazide. Initial results indicate a significant difference in stereoselectivity between the two peaks, the peak attributed to chymopapain exhibiting the greater stereoselectivity.

Relative Reactivities of Reactants

The mandelic hydrazides were very effective nucleophiles, exhibiting no apparent difficulty in entering the S′-subsite region (*29, 30*) and attacking the thioester intermediate. Conversely, (*RS*)-atrolactic hydrazide did not display such facility in the formation of product. Initial trials with (*RS*)-atrolactic hydrazide and *Z*-glycine yielded no insoluble product over extended incubation periods. To achieve a measurable amount of product with racemic atrolactic hydrazide as the nucleophile, the enzyme concentration was doubled. This additional enzyme level gave rise to a satisfactory yield of insoluble N^1,N^2-diacylhydrazine. Possibly the extra methyl group on (*RS*)-atrolactic hydrazide encounters some additional steric hindrance at this region of the active site.

For a given nucleophile, the nature of the *N*-blocked amino acid substrate

determined the rate and extent of product formation. Early experiments with AOC- and BOC-glycine as electrophiles were ineffective in producing insoluble diacylhydrazines with (*RS*)-mandelic hydrazide as the nucleophile. These same *N*-acylamino acids, however, had previously been able to acylate the crude enzyme when allowed to react with aniline and the three anisidines (*31*). In a considerably reduced volume of buffer, with the same quantities of other solution components, desired N^1,N^2-diacylhydrazines were obtained. Early incubation periods gave rise to minute amounts of product; however, after incubation for several days, substantial product was secured. Nevertheless, the yield of products was much less when AOC- and BOC-glycine were used, when compared to the other *N*-blocked amino acids employed. In an effort to obtain a higher yield, the concentration of crude enzyme was increased. This change did not promote any apparent increase in the extent or rate of product formation, indicating the rate is not limited by an enzyme catalyzed step. Both *Z*-glycine and hippuric acid displayed approximately equal abilities to produce insoluble N^1,N^2-diacylhydrazines. When *Z*-L-alanine was the electrophile, the rate and yield of product formation were much greater than with any of the other *N*-blocked amino acids. Initial attempts, under the same conditions as described for *Z*-glycine and hippuric acid, gave nearly a 50% yield during the first 2 hr of incubation. This behavior posed a problem in determining the extent of stereoselective action, since it is desirable to accumulate relatively small quantities of product over extended incubation periods. By reducing the enzyme concentration to one-sixth the amount used in other reactions, the formation of product slowed down sufficiently for an effective analysis.

In comparison with the use of hydrazides of *N*-acylamino acids as nucleophiles (*2*), both mandelic and atrolactic hydrazides gave relatively low yields of N^1,N^2-diacylhydrazines.

EXPERIMENTAL

The method for the isolation and activation of crude papain has been previously described (*1*). The activity of the isolated crude papain was assayed by a slightly modified procedure (*1*) of the method by Bergmann and Frankel-Conrat (*32*).

Preparation of Mandelic and Atrolactic Hydrazides

Synthesis of both (*R*)- and (*S*)-methyl mandelates and methyl (*RS*)-atrolactates involved a catalytic dehydrator procedure (*1, 26*). The hydrazides were prepared by subjecting each methyl ester to reaction with hydrazine (98.5%) dissolved in spectrograde methanol (*1, 2*).

General Procedure for Reactions between Acylated Crude Papain and (R)-, (S)-, and (RS)-Mandelic Hydrazides

A 2.4923-g sample of a given mandelic hydrazide (0.015 mol) was combined with an equimolar amount of *N*-blocked amino acid in a mortar and ground to a

fine powder. The mixture was then dissolved in 125 ml of acetate buffer (0.5 *M*, pH 4.5) and filtered through a medium porosity sintered glass funnel. Active, crude papain, 0.3000 g, was added to an equal quantity of L-cysteine · HCl · H_2O and pulverized in a mortar. The mixture was subsequently dissolved in 25 ml acetate buffer (0.5 *M*, pH 4.5) and filtered through a medium porosity sintered glass funnel. The filtrates were combined in a 250-ml glass-stoppered flask and incubated at 40°C. At successive time intervals, the products were collected by suction filtration and washed with 250 ml distilled water. The products obtained for each incubation period were dried at room temperature for 24 hr, then over P_2O_5 for several days. Comparative data are shown in Tables 2 and 3. Periods of incubation, weights of product, significant modifications of the above procedure, and other pertinent information are listed below according to reactants.

Z-Glycine and (RS)-mandelic hydrazide. 0–3 hr, 0.6183 g, mp 152–155°, $[\alpha]_D^{25}$ +6.6°; 3–6 hr, 0.6755 g, mp 153–156°, $[\alpha]_D^{25}$ +5.4°; 6–9 hr, 0.2629 g, mp 155–157°, $[\alpha]_D^{25}$ +4.4°; 9–24 hr, 0.4814 g, mp 156–159°, $[\alpha]_D^{25}$ +2.9°; 24–48 hr, 0.0540 g, mp 157–159°, $[\alpha]_D^{25}$ 0.0°; 48–120 hr, 0.0479 g, mp 157–159°, $[\alpha]_D^{25}$ 0.0°; %N: Calcd 11.76, found 11.74. See Table 2.

Z-Glycine and (R)-mandelic hydrazide. 0–3 hr, 0.6803 g; 3–6 hr, 0.7361 g; 6–9 hr, 0.2794 g; 9–24 hr, 0.4698 g; 24–48 hr, 0.0513 g; 48–120 hr, 0.0496 g; %N: Calcd 11.76, found 11.95. See Table 3.

Z-Glycine and (S)-mandelic hydrazide. 0–3 hr, 0.5788 g; 3–6 hr, 0.6250 g; 6–9 hr, 0.2503 g; 9–24 hr, 0.4244 g; 24–48 hr, 0.0301 g; 48–120 hr, 0.0355 g; %N: Calcd 11.76, found 11.72. See Table 3.

Hippuric acid and (RS)-mandelic hydrazide. 0–3 hr, 0.4509 g; 3–6 hr, 0.5581 g; 6–9 hr, 0.2495 g; 9–24 hr, 0.6644 g; 24–48 hr, 0.1500 g; 48–120 hr, 0.0158 g; mp 217–218°; %N: Calcd 12.84, found 12.79. See Table 2.

Hippuric acid and (R)-mandelic hydrazide. 0–3 hr, 0.4341 g; 3–6 hr, 0.5460 g;

TABLE 3

PURE N^1,N^2-DIACYLHYDRAZINES PRODUCED IN REACTIONS OF ACYLATED CRUDE PAPAIN WITH (*R*)- AND (*S*)-MANDELIC HYDRAZIDES[a]

Product	Melting point (°C)	Yield (%)	$[\alpha]_D^{25}$ in pyridine (°)
Z-Gly-NHNH-(*R*)-Mand	146–147	42.4	+24.1
Z-Gly-NHNH-(*S*)-Mand	146–147	36.5	−23.4
Hipp-NHNH-(*R*)-Mand	204–205	43.3	+20.1
Hipp-NHNH-(*S*)-Mand	204–205	44.1	−20.7
AOC-Gly-NHNH-(*R*)-Mand	209–210	33.2	+83.8
AOC-Gly-NHNH-(*S*)-Mand	209–210	25.3	−83.6
BOC-Gly-NHNH-(*R*)-Mand	203–204	31.4	+79.9
BOC-Gly-NHNH-(*S*)-Mand	203–204	25.3	−80.3
Z-L-Ala-NHNH-(*R*)-Mand	183–185	42.4	−29.8
Z-L-Ala-NHNH-(*S*)-Mand	180–182	37.6	−69.6

[a] See Experimental for reaction conditions.

6–9 hr, 0.2518 g; 9–24 hr, 0.6590 g; 24–48 hr, 0.1455 g; 48–120 hr, 0.0180 g; %N: Calcd 12.84, found 13.01. See Table 3.

Hippuric acid and (S)-mandelic hydrazide. 0–3 hr, 0.4461 g; 3–6 hr, 0.5756 g; 6–9 hr, 0.2501 g; 9–24 hr, 0.6412 g; 24–48 hr, 0.1630 g; 48–120 hr, 0.0090 g; %N: Calcd 12.84, found 12.78. See Table 3.

AOC-Glycine and (RS)-mandelic hydrazide. 0–18 hr, 0.0800 g, mp 234–236°, $[\alpha]_D^{25}$ +28.6°; 18–24 hr, 0.0630 g, mp 228–230°, $[\alpha]_D^{25}$ +23.8°; 24–48 hr, 0.1712 g, mp 223–225°, $[\alpha]_D^{25}$ +10.3°; 48–72 hr, 0.0919 g, mp 220–223°, $[\alpha]_D^{25}$ +9.7°; 72–120 hr, 0.3450 g, mp 211–213°, $[\alpha]_D^{25}$ +3.7°; 120–168 hr, 0.2600 g, mp 225–227°, $[\alpha]_D^{25}$ −13.5°; %N: Calcd 12.46, found 12.39. See Table 2.

AOC-Glycine and (R)-mandelic hydrazide. 0–18 hr, 0.1306 g; 18–24 hr, 0.0941 g; 24–48 hr, 0.1855 g; 48–72 hr, 0.0864 g; 72–120 hr, 0.3788 g; 120–168 hr, 0.2790 g; %N: Calcd 12.46, found 12.26. See Table 3.

AOC-Glycine and (S)-mandelic hydrazide. 0–18 hr, 0.000 g; 18–24 hr, 0.0344 g; 24–48 hr, 0.1033 g; 48–72 hr, 0.0807 g; 72–120 hr, 0.3094 g; 120–168 hr, 0.2111 g; %N: Calcd 12.46, found 12.42. See Table 3.

BOC-Glycine and (RS)-mandelic hydrazide. 0–18 hr, 0.0668, mp 234–236°, $[\alpha]_D^{25}$ +15.8°; 18–24 hr, 0.0275 g, mp 229–231°, $[\alpha]_D^{25}$ +15.1°; 24–48 hr, 0.1461 g, mp 225–227°, $[\alpha]_D^{25}$ +13.4°; 48–72 hr, 0.0967 g, mp 213–215°, $[\alpha]_D^{25}$ +7.2°; 72–120 hr, 0.3805 g, mp 205–207°, $[\alpha]_D^{25}$ +3.8°; 120–168 hr, 0.2311 g, mp 214–216°, $[\alpha]_D^{25}$ −3.0°; %N: Calcd 13.00, found 13.15. See Table 2.

BOC-Glycine and (R)-mandelic hydrazide. 0–18 hr, 0.0759 g; 18–24 hr, 0.0333 g; 24–48 hr, 0.1560 g; 48–72 hr, 0.0839 g; 72–120 hr, 0.3566 g; 120–168 hr, 0.2109 g; %N: Calcd 13.00, found 12.87. See Table 3.

BOC-Glycine and (S)-mandelic hydrazide. 0–18 hr, 0.0311 g; 18–24 hr, 0.0196 g; 24–48 hr, 0.1134 g; 48–72 hr, 0.0849 g; 72–120 hr, 0.3566 g; 120–168 hr, 0.2109 g; %N: Calcd 13.00, found 12.92. See Table 3.

Z-L-Alanine and (RS)-mandelic hydrazide. 0–1 hr, 0.8985 g, mp 175–178°, $[\alpha]_D^{25}$ −40.8°; 1–3 hr, 2.1834 g, mp 176–178°, $[\alpha]_D^{25}$ −41.5°; 3–4 hr, 0.0884 g, mp 175–178°, $[\alpha]_D^{25}$ −45.6°; 4–5 hr, 0.0336 g, mp 176–178°, $[\alpha]_D^{25}$ −45.9°; 5–7 hr, 0.1202 g, mp 176–178°, $[\alpha]_D^{25}$ −46.4°; 7–24 hr, 0.2912 g, mp 175–178°, $[\alpha]_D^{25}$ −49.8°; %N: Calcd 11.31, found 11.18. See Fig. 2.

Z-L-Alanine and (R)-mandelic hydrazide. 0–2 hr, 1.5495 g; 2–4 hr, 0.5205 g; 4–24 hr, 1.7609 g; $[\alpha]_D^{25}$ −29.8°; %N: Calcd 11.31, found 11.24. See Table 3.

Z-L-Alanine and (S)-mandelic hydrazide. 0–2 hr, 1.2805 g; 2–4 hr, 0.4951 g; 4–24 hr, 1.624 g; $[\alpha]_D^{25}$ −69.6°; %N: Calcd 11.31, found 11.20. See Table 3.

General Procedure for Reactions between Acylated Crude Papain and (RS)-Atrolactic Hydrazide

A 2.5431-g sample of (*RS*)-atrolactic hydrazide (0.015 mol) was added to 0.015 mol of *N*-blocked amino acid. The mixture was ground to a fine powder in a mortar, dissolved in 125 ml of acetate buffer (0.5 *M*, pH 4.5), and filtered through a medium porosity sintered glass funnel. A 0.6000-g sample of crude papain was mixed with 0.6000 g L-cysteine · HCl · H_2O, ground in a mortar, dissolved in 25 ml acetate buffer (0.5 *M*, pH 4.5), and filtered through a medium porosity sintered

glass funnel. The two filtrates were combined in a 250-ml glass-stoppered flask and incubated at 40°C. Product was removed at monitored time intervals, washed with 250 ml distilled water, and dried over P_2O_5. Pertinent information is listed below according to reactants.

Z-Glycine and (RS)-atrolactic hydrazide. 0–4 hr, 0.1652 g, mp 160–165°, $[\alpha]_D^{25}$ +4.5°; 4–8 hr, 0.1307 g, mp 160–164°, $[\alpha]_D^{25}$ +3.4°; 8–24 hr, 0.0515 g, mp 160–162°, $[\alpha]_D^{25}$ +2.1°; 24–48 hr, 0.0081 g, mp 160–163°; %N: Calcd 11.39, found 11.39.

Hippuric acid and (RS)-atrolactic hydrazide. 0–4 hr, 0.1431 g, mp 189–193°, $[\alpha]_D^{25}$ +3.1°; 4–8 hr, 0.1008 g, mp 189–192°, $[\alpha]_D^{25}$ +2.2°; 8–24 hr, 0.0677 g, mp 190–192°, $[\alpha]_D^{25}$ +1.4°; 24–48 hr, 0.0193 g, mp 189–191°; %N: Calcd 12.34, found 12.11.

AOC-Glycine and (RS)-atrolactic hydrazide. 0–4 hr, 0.000 g; 4–24 hr, 0.1134 g, mp 194–197°, $[\alpha]_D^{25}$ +6.9°; 24–48 hr, 0.0874 g, mp 194–196°, $[\alpha]_D^{25}$ +3.9°; 48–72 hr, 0.000 g; %N: Calcd 11.96, found 11.92.

BOC-Glycine and (RS)-atrolactic hydrazide: No reaction observed.

Attempted Reactions of Racemic Mandelic and Atrolactic Hydrazides as Sole Substrates

Active crude papain, 0.3000 g, and an equal weight of L-cysteine · HCl · H_2O were dissolved in 30 ml of acetate buffer (0.5 *M*, pH 4.5). The solution was filtered through a sintered glass funnel. A 1.000-g sample of the racemic hydrazide was dissolved in 70 ml of acetate buffer (0.5 *M*, pH 4.5), filtered through a sintered glass funnel, combined with the filtered crude papain solution, and incubated at 40°C. After 7 days of incubation, no product was observed for either racemic hydrazide.

Preparation of Mixtures of N^1-(Z-L-Alanyl)-N^2-[(R)-Mandelyl]Hydrazine and N^1-(Z-L-Alanyl)-N^2-[(S)-Mandelyl]Hydrazine for Standard Curve (Fig. 2)

25% N^1-(Z-L-Alanyl)-N^2-[(S)-Mandelyl]Hydrazine

A 0.0250-g sample of N^1-(Z-L-alanyl)-N^2[(S)-mandelyl]hydrazine was added to 0.0750 g of N^1(Z-L-alanyl)-N^2[(R)-mandelyl]hydrazine dissolved in 10.00 ml of spectrograde pyridine. $[\alpha]_D^{25}$ −38.8 ± 0.3°.

50% N^1-(Z-L-Alanyl)-N^2-[(S)-Mandelyl]Hydrazine

A 0.0500-g sample of N^1-(Z-L-alanyl)-N^2-[(S)-mandelyl]hydrazine was added to an equal weight of N^1-(Z-L-alanyl)-N^2-[(R)-mandelyl]hydrazine and dissolved in 10.00 ml of spectrograde pyridine $[\alpha]_D^{25}$ −49.5 ± 0.3°.

75% N^1-(Z-L-Alanyl)-N^2-[(S)-Mandelyl]Hydrazine

A 0.0750-g sample of N^1-(Z-L-alanyl)-N^2-[(S)-mandelyl]hydrazine was added to 0.0250 g N^1-(Z-L-alanyl)-N^2-[(R)-mandelyl]hydrazine and dissolved in 10.00 ml of spectrograde pyridine. $[\alpha]_D^{25}$ −59.1 ± 0.5°.

Ion-Exchange Chromatography of Crude Papain

A 0.5000-g sample of active, crude papain was dissolved in 5.0 ml acetate buffer (0.02 *M*, pH 5.0) and added to a column (2.0 × 30 cm) containing Sephadex CM-50, equilibrated with acetate buffer (0.02 *M*, pH 5.0) at 4°C. The column was eluted with five 100-ml volumes of pH 5.0 acetate buffer of increasing ionic strength. The buffer concentrations used were 0.02, 0.10, 0.35, 0.70, and 1.0 *M*. Fractions of 3 ml were collected and absorbance at 280 nm was measured.

Polarimetric Measurements

High optical rotations were measured in a 1-dm water-jacketed polarimeter tube by means of a Rudolph Model 80 high precision polarimeter at 25°, D-line sodium, $C = 0.01$ g/ml in spectrograde pyridine. Lower observed values were measured similarly with a Perkin–Elmer Model 243 polarimeter.

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