[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FRESNO STATE COLLEGE]

Resolutions and an Attempted Partial Asymmetric Synthesis in Papain-Catalyzed Syntheses of N^{α},N^{β}-Diacylhydrazines from Hydrazides and Acylated Amino Acids

BY JOHN LEO ABERNETHY,[1] MARVIN KIENTZ,[2] RONALD JOHNSON AND RODNEY JOHNSON

RECEIVED AUGUST 19, 1958

N^{α},N^{β}-Diacylhydrazines have been prepared successfully from hydrazides and acylated amino acids by papain catalysis. In every reaction where racemic acylated amino acids were employed, resolution occurred. The optimum *p*H for the reaction between benzhydrazide and hippuric acid was about 4. Twenty-six new diacylhydrazines were prepared. In the reaction between DL-mandelic hydrazide and hippuric acid no resolution occurred, but a racemic product resulted. An attempted partial asymmetric synthesis in the reaction between ethylmalonic hydrazide and carbobenzoxyglycine was not successful, but this reaction likewise gave a racemic product.

Since the original work of Bergmann and Fraenkel-Conrat[3] on papain-catalyzed reactions between acylated amino acids and either aniline or phenylhydrazine, much work has been done in extending these reactions to other acylated amino acids[4–8] and even substituted anilines.[9,10] The present investigation extends this reaction to hydrazides. There were five main objectives in this study: (1) to establish the dependence of yield on *p*H for the reaction between benzhydrazide and hippuric acid as a model for subsequent reactions; (2) to determine the effect of variation and location of substituent on substituted benzhydrazides in these reactions; (3) to resolve racemic acylated amino acids by means of papain in reactions with hydrazides; (4) to attempt to resolve DL-mandelic hydrazide in its reaction with hippuric acid, a non-asymmetric acylated amino acid; (5) to attempt a partial asymmetric synthesis in the reaction between ethylmalonic hydrazide and carbobenzoxyglycine.

The dependence of yield on *p*H for the formation of N^{α}-benzoyl-N^{β}-hippurylhydrazine from benzhydrazide and hippuric acid is shown in Fig. 1. Nearly all of the subsequent reactions were carried out at *p*H approximately 4.0. Other substituted benzhydrazides employed were: salicylic hydrazide, *m*- and *p*-hydroxybenzhydrazides; *o*-, *m*- and *p*-toluic hydrazides; and *o*-, *m*- and *p*-nitrobenzhydrazides. With other acylated amino acids, such as carbobenzoxy-DL-alanine, carbobenzoxy-L-alanine, benzoyl-DL-alanine and benzoyl-L-alanine, usually N^{α},N^{β}-diacylhydrazines were formed.

$$XC_6H_4CONHNH_2 + HOCOCHRNHCOR' \xrightarrow[40°,\ pH\ 4.0]{\text{papain, L-cysteine}} XC_6H_4CONHNHCOCHRNHCOR' + H_2O$$

X = –OH, $-CH_3$ or $-NO_2$; R = –H or $-CH_3$; R′ = $-C_6H_5$ or $-OCH_2C_6H_5$

DL-Mandelic hydrazide contains an asymmetric carbon. Its reactions with non-asymmetric hippuric acid was of particular interest because of the possibility of resolution during the formation of N^{α}-hippuryl-N^{β}-mandelylhydrazine, under the catalytic action of papain.

$$C_6H_5CHOHCONHNH_2 + HOCOCH_2NHCOC_6H_5 \xrightarrow[40°,\ pH\ 4.0]{\text{papain, L-cysteine}} C_6H_5CHOHCONHNHCOCH_2NHCOC_6H_5 + H_2O$$

Furthermore, if papain could direct the attack of carbobenzoxyglycine on ethylmalonic hydrazide toward a particular hydrazide group, a partial asymmetric synthesis would result, as for example

$$\underset{\text{ethylmalonic hydrazide (non-asymmetric)}}{C_2H_5CH(CONHNH_2)_2} + HOCOCH_2NHCOOCH_2C_6H_5 \xrightarrow[40°,\ pH\ 4.0]{\text{papain, L-cysteine}} \underset{N^{\beta}\text{-(benzyloxycarbonylaminoacetyl)-2-ethylmalonohydrazide (asymmetric and optically active)}}{C_2H_5CH(CONHNHCOCH_2NHCOOCH_2C_6H_5)CONHNH_2} + H_2O$$

Such an attack has long been known to be a very reasonable possibility.[11,12]

Experimental

Preparation of Hydrazides.—For the preparation of the hydrazides an excess of 95% hydrazine was refluxed for several hours with either the methyl or the ethyl ester of the corresponding acid in a ratio of about 1.5 moles of hydrazine to 1 mole of ester. Recrystallization from water or ethyl alcohol usually gave a product that was sufficiently pure for use in subsequent experiments. With *o*-nitrobenzhydrazide, equal molar quantities of methyl *o*-nitrobenzoate and 95% hydrazine were warmed for about 2 hours on a water-bath. Cooling yielded the solid hydrazide, which was recrystallized from ethanol. Water was avoided because of the high solubility of this hydrazide in water. Ethylmalonic hydrazide was prepared by warming ethyl ethylmalonate with a slight excess of 95% hydrazine on a water-bath for about 4 hours. Cooling produced the solid hydrazide, which was removed by filtration and recrystallized from ethanol.

Synthesis of Carbobenzoxy-DL-alanine, Carbobenzoxy-L-alanine and Carbobenzoxyglycine.—These three acylated

(1) To whom inquiries concerning this paper should be directed.

(2) Fresno County Heart Association Undergraduate Fellow, 1957–1958; California Heart Association Undergraduate Research Award, 1957.

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

(4) J. S. Fruton, G. W Irving, Jr., and Max Bergmann, *ibid.*, **133**, 703 (1940).

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

(6) E. L. Bennett and C. Niemann, *ibid.*, **72**, 1798 (1950).

(7) S. W. Fox and H. Wax, *ibid.*, **72**, 5087 (1950).

(8) D. G. Doherty and E. A. Popenoe, Jr., *J. Biol. Chem.*, **189**, 447 (1951).

(9) E. Waldschmidt-Leitz and K. Kuhn, *Z. physiol. Chem.*, **285**, 23 (1950).

(10) J. L. Abernethy, J. Nakamura and Bro. Myron Collins, *J. Org. Chem.*, **23**, 586 (1958).

(11) A. G. Ogston, *Nature*, **162**, 963 (1948).

(12) H. Hirschmann, in S. Graff, "Essays in Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1956, p. 156.

amino acids were prepared according to the method described in reference 13 from benzyl chloroformate and one of the amino acids: DL-alanine, L-alanine or glycine.

Activation of Papain.[14]—The method of activation of papain was essentially that given by Abernethy, Nakamura and Collins.[10] One important modification was used. When filtration proved to be difficult, just after dissolving the commercial, unactivated papain and particularly right after passing hydrogen sulfide into soluble papain, the filtration was preceded by centrifuging for 20 minutes at 2000 r.p.m., twice if necessary. The solution was then decanted onto a suction filter and filtration took place more rapidly.

Dependence of Yield on *p*H for the Reaction between Hippuric Acid and Benzhydrazide to Form N^{α}-Benzoyl-N^{β}-hippurylhydrazine.—To each of eight flasks was added 0.5000 g. of L-cysteine hydrochloride, 1.7917 g. of hippuric acid and 1.3614 g. of benzhydrazide. The appropriate buffer was added in sufficient quantity to dissolve these solid reagents when hot. The solutions were filtered. Then 2.500 g. of activated Wallerstein papain was dissolved in about 5 ml. of cold buffer. This amount of dissolved papain was rinsed into each of the filtered solutions with several portions of the filtered solutions, after they had been cooled to a temperature below 40°. Each resultant solution was made up to a total of about 120 ml. with the addition of more buffer. The *p*H of each solution was adjusted to the exact *p*H desired, with the aid of a *p*H meter. Finally, sufficient more buffer was added to make the total volume exactly 125 ml. The solutions were incubated at 40°. Precipitates of N^{α}-benzoyl-N^{β}-hippurylhydrazine were collected at the end of 12, 24 and 36 hr., dried and weighed. The data are given graphically in Fig. 1.

Since the optimum *p*H for these experimental conditions of this reaction proved to be about 4.0, this *p*H was used in nearly all experiments.

N^{α}-Benzoyl-N^{β}-hippurylhydrazine from a More Concentrated Solution of Reactants.—A buffered solution, *p*H ≦ 4.3–4.5, was made up to a total volume of 250 ml. and contained 0.0500 mole of benzhydrazide, 0.0500 mole of hippuric acid, 1.000 g. of L-cysteine hydrochloride and 0.5000 g. of activated Schwarz papain. The solution was filtered and then incubated at 40° with these yields of product after the designated periods of incubation: 0–18 hr., 13.1000 g.; 18–42 hr., 0.0096 g.; 42–66 hr., 0.0013 g.; 66–90 hr., 0.0003 g.; 90–114 hr., 0.0005 g.; 114–138 hr., 0.0008 g.; 138–162 hr., 0.0006 g.; 162–330 hr., 0.1074 g. A yield of 92% was given in the first 18 hr. Recrystallization from ethanol yielded a product melting at 219–220.5° (Mr. Calvin Johnson performed this experiment.)

The Attempted Resolution of DL-Mandelic Hydrazide by its Papain-catalyzed Reaction with Hippuric Acid to Form N^{α}-Hippuryl-N^{β}-mandelylhydrazine.—DL-Mandelic hydrazide, 0.0200 mole, and 0.0200 mole of hippuric acid were dissolved in 220 ml. of buffer, *p*H ≦ 4.0 and 1.000 g. of L-cysteine hydrochloride was then added. The mixture was warmed to dissolve the substances and then cooled to 40°. The *p*H was adjusted to 4.0. Papain, 0.500 g., was ground in a mortar with 15 ml. of new buffer solution and then added to these reactants. Two 7-ml. portions of new buffer solution were used to rinse the mortar and pestle and the washings were added to the reaction flask. The resultant solution was filtered, stoppered and incubated at 40°. At the end of 12, 24, 36 and 168 hr. the solid reaction product was removed by filtration: 0–12 hr., 1.3518 g.; 12–24 hr., 0.8316 g.; 24–36 hr., 0.0585 g.; 36–168 hr., 0.1382 g. After recrystallization from ethanol the melting point was consistently 211–212° and the rotation in pyridine was 0.000° in each case.

Anal. Calcd. for $C_6H_5CONHCH_2CONHNHCOCHOHC_6H_5$: N, 12.84. Found: N, 12.95.

The Attempted Partial Asymmetric Synthesis of an Optically Active N^{β}-(Benzyloxycarbonylaminoacetyl)-2-ethylmalonohydrazide from the Papain-catalyzed Reaction between Ethylmalonic Hydrazide and Carbobenzoxyglycine.—A mixture of 5.200 g. of carbobenzoxyglycine, 4.000 g. of ethylmalonic hydrazide, 0.5000 g. of L-cysteine hydrochloride and 1.0000 g. of activated Wallerstein papain was made up to a total volume of 150 ml. and the *p*H was adjusted to 4.0 and the solution was filtered. Incubation was carried out at 40° and these quantities of N^{β}-(benzyloxycarbonylaminoacetyl)-2-ethylmalonohydrazide were obtained: 0–12 hr., 6.8063 g.; 12–24 hr., trace; 24–36 hr., 0.0000 g.; 36–168 hr., 0.0247 g. The melting point of the product from the 0–12-hr. period of incubation, after recrystallization from ethanol, was 210–211° and its rotation in pyridine was 0.000°.

Anal. Calcd. for $NH_2NHCOCH(C_2H_5)CONHNHCOCH_2NHCOOCH_2C_6H_5$: N, 19.93. Found: N, 19.70.

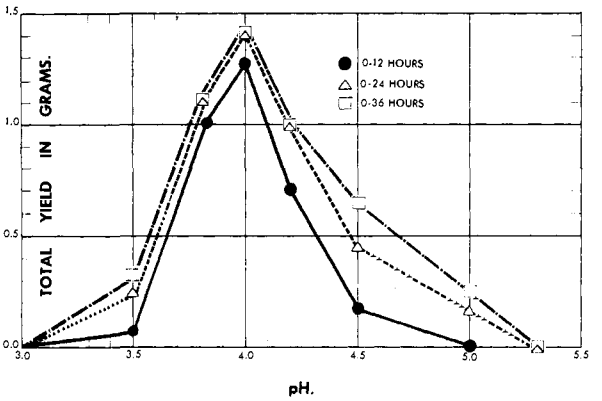


Fig. 1.—Dependence of yield on *p*H for the papain-catalyzed synthesis of N^{α}-benzoyl-N^{β}-hippurylhydrazine from benzhydrazide and hippuric acid at 40°.*

* Total volume of buffered solution was 125 ml. and contained: 0.0100 mole of benzhydrazide, 0.0100 mole of hippuric acid, 0.5000 g. of L-cysteine hydrochloride and 0.2500 g. of activated Wallerstein papain.

A Comparison of Papain-catalyzed Reactions between Benzhydrazide and Substituted Benzhydrazides with Acylated Amino Acids.—Each of the hydrazides, benzhydrazide, salicylic hydrazide, *m*- and *p*-hydroxybenzhydrazides, *o*-, *m*- and *p*-nitrobenzhydrazides, *o*-, *m*- and *p*-toluic hydrazides was treated with hippuric acid, carbobenzoxy-DL-alanine, carbobenzoxy-L-alanine, benzoyl-DL-alanine and benzoyl-L-alanine.

The general procedure was to employ 0.0100 mole of the hydrazide and 0.0100 mole of one of the acylated amino acids if it was hippuric acid or an L-isomer, and 0.0200 mole of the acylated amino acid if it was racemic. Then 0.500 g. of L-cysteine hydrochloride and 0.250 g. of activated Wallerstein papain were employed. The solution was made up nearly to 150 ml. with buffer of *p*H 4.0, and the *p*H adjusted exactly to 4.0 and enough more buffer added to make the total volume exactly 150 ml. Care was taken never to heat the papain above 40°.

Certain variations were necessary. When carbobenzoxy-DL-alanine was employed with the hydroxybenzhydrazides, the total volume of solution was always 250 ml. For all reactions of *p*-hydroxybenzhydrazide the total volume of solution was 250 ml. due to the insolubility of this compound, and there was considerable insoluble substrate in each case, which was removed by filtration before incubation was begun. With *m*-toluic hydrazide and carbobenzoxy-DL-alanine, a small amount of insoluble substrate had to be removed before incubation was started. When *m*-nitrobenzhydrazide was employed with hippuric acid, a certain amount of insoluble substrate was removed before incubation was started. *p*-Nitrobenzhydrazide was not as soluble as the other two nitro derivatives, so that 250-ml. total solution was used. In each case a certain amount of insoluble substrate was removed before incubation was started.

At the end of 12, 24, 36 and 168 hr., insoluble diacylhydrazines were removed by filtration, dried and weighed. *p*H adjustment to 4.0 was made after each filtration before incubation was continued. The diacylhydrazines were recrystallized by dissolving in hot methanol or hot ethanol, treating with carbon, filtering three times, pouring into cold

(13) H. E. Carter, R. L. Frank and H. W. Johnson in E. C. Horning, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 168.

(14) The papain was generously supplied by the Wallerstein Laboratories, New York City, and the Schwarz Laboratories, Mount Vernon, N. Y.

TABLE 1

N^{α},N^{β}-DIACYLHYDRAZINES FROM HYDRAZIDES AND ACYLATED AMINO ACIDS AT *p*H 4.0

Reactants: hydrazides with acylated amino acids[a]	Hydrazine product RCONHNHCOR′	Nitrogen, % Calcd.	Found	M.p., °C.	$[\alpha]^{25}D$ in pyridine
Benzhydrazide with					
HA[a]	N^{α}-Benzoyl-N^{β}-hippuryl-	14.14	14.05	219–220	
C-L-A[a]	N^{α}-Benzoyl-N^{β}-carbobenzoxy-L-alanyl-	12.40	12.29	204–205	−38.31°
C-DL-A[a]	N^{α}-Benzoyl-N^{β}-carbobenzoxyalanyl-			203–204	−35.78
B-DL-A[a]	N^{α}-Benzoyl-N^{β}-benzoylalanyl-	13.50	13.35	219–220	−45.05
Salicylic hydrazide with					
HA	N^{α}-Hippuryl-N^{β}-salicyl-	13.41	13.67	255–256	
C-L-A	N^{α}-Carbobenzoxy-L-alanyl-N^{β}-salicyl-	11.76	11.87	197–198.5	−55.90
C-DL-A	N^{α}-Carbobenzoxyalanyl-N^{β}-salicyl-			190–191.5	−55.87
B-DL-A	N^{α}-Benzoylalanyl-N^{β}-salicyl-	12.84	12.55	204–205.5	−57.98
m-Hydroxybenzhydrazide with					
HA	N^{α}-Hippuryl-N^{β}-*m*-hydroxybenzoyl-	13.41	13.19	259–261	
C-L-A	N^{α}-Carbobenzoxy-L-alanyl-N^{β}-*m*-hydroxybenzoyl-	11.76	11.55	178–179.5	−36.77
C-DL-A	N^{α}-Carbobenzoylalanyl-N^{β}-*m*-hydroxybenzoyl-			198–201	− 4.69
B-DL-A	N^{α}-Benzoylalanyl-N^{β}-*m*-hydroxybenzoyl-	No reaction			
p-Hydroxybenzhydrazide	No reaction with HA, C-L-A, C-DL-A or B-DL-A				
o-Toluic hydrazide with					
HA	No reaction				
C-L-A	N^{α}-Carbobenzoxy-L-alanyl-N^{β}-*o*-toluyl-	11.83	12.00	207–209	−51.01
C-DL-A	N^{α}-Carbobenzoxyalanyl-N^{β}-*o*-toluyl-			202–203	−42.00
B-DL-A	N^{α}-Benzoylalanyl-N^{β}-*o*-toluyl-	12.92	13.08	182–185	−42.80
m-Toluic hydrazide with					
HA	N^{α}-Hippuryl-N^{β}-*m*-toluyl-	13.50	13.35	212–214	
C-L-A	N^{α}-Carbobenzoxy-L-alanyl-N^{β}-*m*-toluyl-	11.83	11.93	180–182	−51.30
C-DL-A	N^{α}-Carbobenzoxyalanyl-N^{β}-*m*-toluyl-			202–204	−51.20
B-DL-A	N^{α}-Benzoylalanyl-N^{β}-*m*-toluyl-	12.92	12.69	175–177	−53.87
p-Toluic hydrazide with					
HA	N^{α}-Hippuryl-N^{β}-*p*-toluyl-	13.50	13.71	226–228	
C-L-A	N^{α}-Carbobenzoxy-L-alanyl-N^{β}-*p*-toluyl-	11.83	11.69	189–191	−51.52
C-DL-A	N^{α}-Carbobenzoxyalanyl-N^{β}-*p*-toluyl-			188–189	−49.56
B-DL-A	N^{α}-Benzoylalanyl-N^{β}-*p*-toluyl-	12.92	13.09	214–216	−28.16
o-Nitrobenzhydrazide with					
HA	No reaction				
C-L-A	N^{α}-Carbobenzoxy-L-alanyl-N^{β}-*o*-nitrobenzoyl-	14.50	14.63	238–239	−61.2
C-DL-A	N^{α}-Carbobenzoxyalanyl-N^{β}-*o*-nitrobenzoyl-			233–234	−48.7
B-DL-A	N^{α}-Benzoylalanyl-N^{β}-*o*-nitrobenzoyl-	15.72	15.47	252–254	−16.2
m-Nitrobenzhydrazide with					
HA	N^{α}-Hippuryl-N^{β}-*m*-nitrobenzoyl-	16.37	16.48	199–200	
C-L-A	N^{α}-Carbobenzoxy-L-alanyl-N^{β}-*m*-nitrobenzoyl-	14.50	14.77	252–254	−43.8
C-DL-A	N^{α}-Carbobenzoxyalanyl-N^{β}-*m*-nitrobenzoyl-			224–225	−38.1
B-DL-A	N^{α}-Benzoylalanyl-N^{β}-*m*-nitrobenzoyl-	15.72	15.47	238–240	−53.3
p-Nitrobenzhydrazide with					
HA	N^{α}-Hippuryl-N^{β}-*p*-nitrobenzoyl-	16.37	16.61	242–243	
C-L-A	N^{α}-Carbobenzoxy-L-alanyl-N^{β}-*p*-nitrobenzoyl-	14.50	14.62	230–231	−46.3
C-DL-A	N^{α}-Carbobenzoxyalanyl-N^{β}-*p*-nitrobenzoyl-			224–225	−45.0
B-DL-A	N^{α}-Benzoylalanyl-N^{β}-*p*-nitrobenzoyl-	15.72	16.01	254–255	−50.0

[a] Hippuric acid = HA; carbobenzoxy-L-alanine = C-L-A; carbobenzoxy-DL-alanine = C-DL-A; and benzoyl-DL-alanine = B-DL-A.

water, washing the precipitate with hot water and drying over phosphorus pentoxide. In a few instances the alcohol solution was allowed to evaporate, rather than being poured into cold water. Rotations were all taken in pyridine at a concentration of about 2%. The results are given in tabular form. Abbreviations used for the acylated amino acids are: hippuric acid (HA); benzoyl-DL-alanine (B-DL-A;) carbobenzoxy-DL-alanine (C-DL-A); carbobenzoxy-L-alanine (C-L-A). For the hydrazides, these abbreviations are employed: benzhydrazide (BH); salicylic hydrazide (SH); hydroxybenzhydrazide (HBH); toluic hydrazide (TH); nitrobenzhydrazide (NBH).

For the majority of reactions, they were complete, or essentially so, at the end of the first 12 hours of incubation. Yields in these cases for this period were as listed. BH with C-L-A, 2.0398 g.; SH with: C-L-A, 0.6815 g., C-DL-A, 1.2891 g.; *m*HBH with C-L-A-, 0.2833 g.; *o*TH with: C-L-A, 1.6777 g.; C-DL-A, 1.6640 g.; *m*-TH with: B-DL-A, 1.6244 g.; C-L-A, 2.7904 g.; C-DL-A, 2.5462 g.; *p*TH with: B-DL-A, 1.9024 g.; C-L-A, 2.1086 g.; C-DL-A, 1.9024 g.; *o*NBH with: B-DL-A, 1.9595 g.; C-L-A, 2.9200 g.; C-DL-A, 2.7421 g.; *m*NBH with: B-DL-A, 1.8596 g.; C-L-A, 2.2405 g.; C-DL-A, 2.2555 g.; *p*NBH with: C-L-A, 0.9934 g.; C-DL-A, 1.2187 g.

Several reactions gave a considerable quantity of product at the end of the first 12 hours and continued to give substantial amounts after this time. Yields for the first 12 hours were: BH with: HA, 0.2651 g.; C-DL-A, 1.3178 g.; SH with: HA, 0.9802 g.; B-DL-A, 1.5629 g.; *m*TH with HA, 0.4650 g.; *p*TH with HA, 1.2736 g.; *m*NBH with HA, 1.2965 g.; *p*NBH with: HA, 0.4899 g.; B-DL-A, 0.5846 g.

Three reactions gave little or no product during the first 12 hours but gave substantial amounts after this. *m*HBH with HA: 0–12 hr., 0.0000 g.; 12–24 hr., 0.3064 g.; 24–36 hr., 0.4373 g.; BH with B-DL-A: 0–12 hr., 0.0357 g.; 12–24 hr., 1.1956 g.; 24–36 hr., 0.0000 g.; *o*TH with B-DL-A: 0–12 hr., 0.0189 g.; 12–24 hr., 1.2501 g.; 24–36 hr., 0.0000 g.

None of the hydrazides gave a reaction with benzoyl-L-alanine when this antipode was not in a racemic mixture but was by itself. Other combinations not listed did not undergo reactions.

Discussion of Results

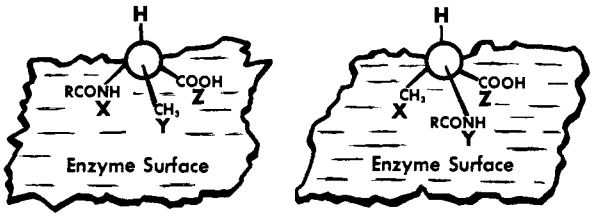
Although the usual *p*H employed for papain-catalyzed reactions between acylated amino acids and aniline, substituted anilines and phenylhydrazine is between 4.5 and 5.0, it was found that a *p*H close to 4.0 appears to be best for the reaction between hippuric acid and benzhydrazide. This is presumably in accord with the difference in basicity of the hydrazide amino group, $-NH_2$, as compared with the amino groups in these other compounds. In general the reaction proceeds much more rapidly with the hydrazides and acylated amino acids than it does with the other amino-containing compounds investigated. Frequently the reaction is essentially finished at the end of the first 12 hours of incubation. When relatively high concentrations of hippuric acid and benzhydrazide were employed, the yield of product was over 90% at the end of this 12-hour period.

Papain proved to be an effective resolving agent in most instances where carbobenzoxy-DL-alanine and benzoyl-DL-alanine were utilized with various hydrazides. This is usually regarded[15] to be a consequence of a three-point contact of the substrate with the enzyme at X, Y and Z (Fig. 2). The acylated L-amino acid usually gives a more satisfactory contact between the activated carboxyl and position Z of the enzyme, than does the D-isomer. Hence Z provides activation of the carbonyl group, while simultaneously X more satisfactorily meets the needs of the acylated amino radical and Y accommodates the methyl radical better. The extent of resolution of carbobenzoxy-DL-alanine could be judged by comparing the rotation of the resultant optically active N^{α},N^{β}-diacylhydrazine with the one resulting from carbobenzoxy-L-alanine. Benzoyl-L-alanine, free from its antipode, did not give a diacylhydrazine with any of the hydrazides employed.

It was not possible to bring about a resolution of DL-mandelic hydrazide by its papain-catalyzed reaction with carbobenzoxyglycine. This might be expected because the mechanism of activation no doubt involves an interaction of papain with the carbonyl group of the carboxyl of an acylated amino acid. It has been established by Smith[16] that the active site of papain for the hydrolysis of amide linkages is at a sulfur atom in the form of a thiolactone (or thioester) produced and maintained

(15) M. Bergmann and J. S. Fruton, *Advances in Enzymol.*, **1**, 63 (1941).

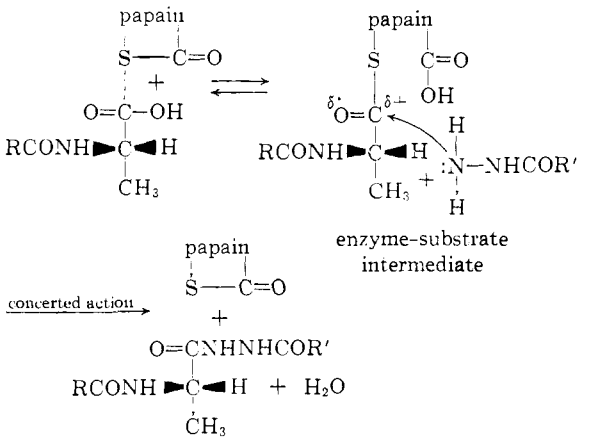
(16) E. L. Smith, *J. Biol. Chem.*, **233**, 1382 (1958).



Acylated L-amino acid on the enzyme surface | Acylated D-amino acid on the enzyme surface

Fig. 2.—Three-point contact of acylated amino acid with the enzyme.

with a high energy bond by a favorable, folded configuration of the enzyme. It is, therefore, kinetically very reactive. The mechanism for the formation of diacylhydrazines would involve this same thiolactone structure of papain.



Preference is usually shown, with a certain spatially fixed arrangement of the enzyme, for the acylated L-amino acid, with the acylated amino group to the left. When hippuric acid is employed with DL-mandelic hydrazide, R becomes C_6H_5 and CH_3 becomes H, while R′ contains the asymmetric center $-CHOH-C_6H_5$. The less intimate contact of a hydrazide with the enzyme than with an acylated amino acid and the enzyme could account for the failure of papain to resolve DL-mandelic hydrazide.

Papain does not always display preference for the L-isomer of a racemic acylated amino acid, but may form a racemic product[8] instead of a preponderance of the L-isomer. The charge distribution around the active center of the enzyme is, of course, also involved. This distribution might not depend so much on the configurations of amino acids of the polypeptide chains of the enzyme as on the alignment of the chains, perhaps accounting for essentially equal activation of antipodes of acylated amino acids in certain cases. It would be expected that papain would activate carbobenzoxyglycine in its reaction with ethylmalonic hydrazide to form N^{β}-(benzyloxycarbonylaminoacetyl)-2-ethylmalonohydrazide. The reason for failure to give a partial asymmetric synthesis would be similar to the failure to resolve DL-mandelic hydrazide.

These papain-catalyzed reactions demonstrate a novel synthesis of N^{α},N^{β}-diacylhydrazines.

Usual chemical procedures[17] employ acylation of hydrazides, diacylation of hydrazine, thermal decomposition of hydrazides and oxidation of hydrazides with iodine. Among the uses of diacylhydrazines have been investigations of dipole moments[18] and apparent energies of N–N bonds.[19] Still another interesting usage of an acylated hydrazine has been the oxidation of N^{α},N^{α}-diphenyl-N^{β}-picrylhydrazine, which forms N^{α},N^{α}-diphenyl-N^{β}-picrylhydrazyl free radicals rather than the anticipated N^{α},N^{α},N^{β},N^{β}-tetraphenyl-N^{β},N^{γ}-dipicryltetrazane. This purple sold free radical is employed as a standard of measurement for magnetic moments.[20,21] Acylation of N^{α},N^{α}-diphenylhydrazine with picryl chloride yields the starting material for the synthesis of this free radical.

Acknowledgments.—Appreciation is expressed for grants-in-aid from the Society of the Sigma Xi and RESA, which took care of costs of some of the analyses and important chemicals. The Rudolph high-precision polarimeter was purchased through a grant-in-aid from the Research Corporation. Generous grants from the Fresno County Heart Association and the California Heart Association provided for an undergraduate research fellowship for Mr. Marvin Kientz, the purchase of equipment and chemicals, the assistance of Messrs. Ronald and Rodney Johnson in synthesizing a few important intermediates and the expense of many of the analyses. Professor John J. Sampson of the University of California Medical School in San Francisco and Chairman of the Research Committee of the California Heart Association and Dr. Robert Maybury of the University of Redlands and Chairman of the Undergraduate Research Sub-committee of the California Heart Association gave invaluable time and assistance with regard to securing this financial aid. Dr. Robert D. Beech, Chairman of the Research Committee of the Fresno County Heart Association, worked patiently in fostering the financial aid provided. Mrs. Joyce Richardson, Executive Director of the Fresno County Heart Association, and Miss Phillis Hecker, Program Consultant of the California Heart Association, provided much help in enabling the grants to be secured. Appropriate systems of nomenclature were outlined by Professor Charles D. Hurd of Northwestern University. Professor Carl Niemann of the California Institute of Technology read through the major portion of the research data and made important comments before the final organization into this paper. Professor Ennis B. Womack, Chairman of the Chemistry Department of Fresno State College, gave valuable assistance in providing certain items of equipment and space for carrying out the project. Student help, other than that of the major research workers, was given by Mr. Calvin Johnson, Mr. Jerome Blank and Mr. Leon Yengoyan. Most of the papain used in this project was supplied generously by the Wallerstein Laboratories of New York City. Some of the papain had been donated previously by the Schwarz Laboratories of Mount Vernon, N. Y. Nitrogen analyses were all performed by the Schwarzkopf Microanalytical Laboratory of Woodside, N. Y.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA]

The Biogenesis of Morphine[1]

BY EDWARD LEETE

RECEIVED JANUARY 5, 1959

Radioactive morphine was obtained when DL-phenylalanine-2-C^{14} or DL-tyrosine-2-C^{14} was fed to *Papaver somniferum* plants. Systematic degradation of the morphine derived from the tyrosine yielded compounds whose activities were compatible with the hypothesis that morphine is formed from two molecules of tyrosine *via* norlaudanosine.

In 1925,[2] Gulland and Robinson suggested that the morphine skeleton is formed in the plant by the cyclization of norlaudanosine (III). The norlaudanosine is produced by a Mannich reaction, in which decarboxylation takes place between 3,4-dihydroxyphenylalanine (I) and 3,4-dihydroxyphenylacetaldehyde (II) which arises by the oxidative decarboxylation of a second molecule of I. Rotation of ring A of III through 180° gives rise to the equivalent structure IV. It is then immediately apparent that two cyclizations and reduction and dehydration of ring A will give rise to the morphine skeleton. This final conversion of IV to morphine (V) has been the subject of considerable discussion.[3] However, it seemed desirable to check the basic biogenetic scheme before becoming too excited about intimate details of this hypothesis. If this scheme is correct, the feeding of 3,4-dihydroxyphenylalanine-2-C^{14} (I) to opium poppies should result in the labeling of morphine on C-9 and C-16 as indicated in Fig. 1.

(1) A preliminary account of part of this work has appeared as a communication: E. Leete, *Chemistry & Industry*, 977 (1958). This investigation was supported by research grants M-2075 and M-2662, from the National Institute of Mental Health, Public Health Service.

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