The Use of Phenylhydrazine and Substituted Phenylhydrazines for Papain-Catalyzed Resolutions of Racemic *N*-(Benzyloxycarbonyl)alanine

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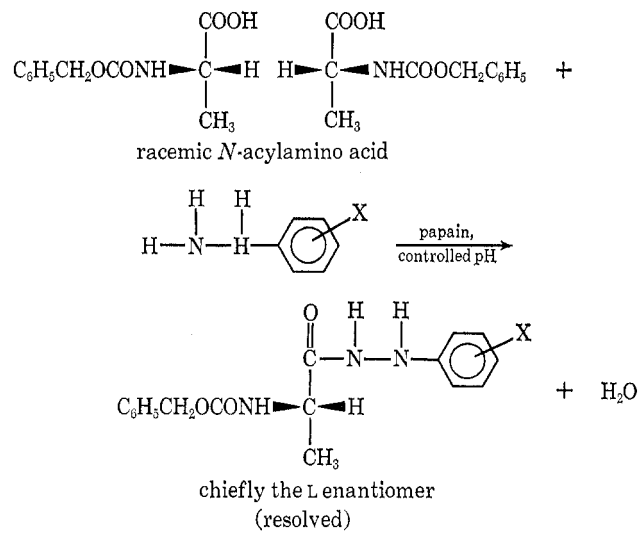
Papain-catalyzed reactions have been carried out between phenylhydrazine, ring-substituted phenylhydrazines, and N^1-methyl-N^1-phenylhydrazine, and four *N*-acylamino acids, namely, hippuric acid, *N*-(benzyloxycarbonyl)-glycine, *N*-(benzyloxycarbonyl)-L-alanine, and N-(benzyloxycarbonyl)-DL-alanine. Thirty-six arylhydrazides were produced. Twelve resolutions of the racemic *N*-acylamino acid were brought about by using different arylhydrazines, due to the chirality of papain. Optical rotations showed that the per cent of L enantiomer varied from about 82 to 99.9%, depending on the arylhydrazine that was used. When the *N*-acylamino acid was *N*-(benzyloxycarbonyl)glycine, the reaction with phenylhydrazine displayed an optimum pH of about 4.0. With N^1-methyl-N^1-phenylhydrazine, the optimum pH was about 4.75.

Interest in resolutions of racemic mixtures and in asymmetric syntheses has been greatly enhanced in recent years because of accelerated attention given to the intricate nature of the origin and perpetuation of life as it exists on earth.[2-4] This report systematically extends studies centered about the behavior of *N*-acylamino acids toward amino-containing bases under papain catalysis. Particular attention was given to the extent of resolution shown by the products from the racemic *N*-acylamino acid that was included for investigation.

The amino bases involved phenylhydrazine, fluorophenylhydrazines, nitrophenylhydrazines, tolylhydrazines, methoxyphenylhydrazines, and N^1-methyl-N^1-phenylhydrazine. All of these were subjected to papain-catalyzed reactions with hippuric acid, *N*-(benzyloxycarbonyl)glycine, *N*-(benzyloxycarbonyl)-L-alanine, and *N*-(benzyloxycarbonyl)-DL-alanine. It was important to determine the optimum pH for the reaction between phenylhydrazine and *N*-(benzyloxycarbonyl)-glycine. A similar determination was made for the reaction between N^1-methyl-N^1-phenylhydrazine and this same *N*-acylamino acid. Other data permit conclusions to be drawn concerning properties of products such as melting points, optical rotations, and relative reaction rates from a qualitative viewpoint.

Resolutions of this racemic *N*-acylamino acid can be represented by the equation shown in column 2. It is well known that the chirality of papain gives preference for the L enantiomer in these reactions through formation of a thio ester[5-7] at the single, existent mercapto group[8] of L-cysteine residue no. 25, when counted from the amino terminal. For this reason, papain has long been classified as a sulfhydryl enzyme.[9] Elucidation of the complete structure of crystalline papain,[10] with its 212 residues, has been achieved through chemical methods[11] and crystallographic analysis.[12]



Results and Discussion

The first experimental work that utilized papain in reversing its proteolytic action was reported by Bergmann and Fraenkel-Conrat[13] in 1937. Both aniline and phenylhydrazine were used with hippuric acid and *N*-(benzyloxycarbonyl)glycine to form the anilides and phenylhydrazides. Although aniline was then used to resolve *N*-(benzyloxycarbonyl)-DL-alanine, nothing else was done to incorporate substituted phenylhydrazines. An optimum pH of 4.0 was found by us for the formation of *N*-(benzyloxycarbonyl)glycine phenylhydrazide, while a pH optimum of 4.75 was exhibited for the production of the corresponding N^1-methyl-N^1-phenylhydrazide. These are shown in Figure 1. All reactions that used ring-substituted phenylhydrazines were buffered at pH 4.0.

The percentage of L enantiomer in the resultant hydrazides can be used as a standard for comparing the degree of resolution of the *N*-acylamino acid. This is given by the familiar equation

$$\text{per cent L enantiomer in } N\text{-acylamino acid phenylhydrazide} = \frac{{}^1/_2([\alpha]_{\text{pure L}} + [\alpha]_{\text{mixture}})}{[\alpha]_{\text{pure L}}} \times 100$$

where $[\alpha]_{\text{pure L}}$ and $[\alpha]_{\text{mixture}}$ are the specific rotations, respectively, of products from the *N*-acyl-L-amino acid and the *N*-acyl-DL-amino acid for each amino base used.

(1) To whom inquiries should be directed.
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TABLE I

PER CENT OF L ENANTIOMER PRESENT IN ARYLHYDRAZIDES FORMED FROM *N*-(BENZYLOXYCARBONYL)-DL-ALANINE BY THE RESOLVING CAPACITY OF PAPAIN DUE TO ITS CHIRALITY

Arylhydrazine reactant	L enantiomer in product, %	Arylhydrazine reactant	L enantiomer in product, %
Phenylhydrazine	88.2	*o*-Tolylhydrazine	91.2
N^1-Methyl-N^1-phenylhydrazine	82.3	*m*-Tolylhydrazine	93.3
o-Methoxyphenylhydrazine	95.7	*p*-Tolylhydrazine	96.3
p-Methoxyphenylhydrazine	88.2	*o*-Fluorophenylhydrazine	99.9
o-Nitrophenylhydrazine	99.1	*m*-Fluorophenylhydrazine	99.0
m-Nitrophenylhydrazine	99.4	*p*-Fluorophenylhydrazine	98.8

Table I shows that the N^1-methyl-N^1-phenylhydrazide contained the lowest percentage of the L enantiomes, while the *o*-fluorophenylhydrazide contained the highest percentage. Substitution with a methyl group on the N^1 position of phenylhydrazine does substantially reduce the percentage of the L enantiomer in the product. No general correlation with other locations of substituents can be made.

In comparing the specific rotations of the arylhydrazides produced from pure *N*-acyl-L-amino acid, it is evident that the N^1-methyl-N^1-phenylhydrazide (−16.4°) has a much lower absolute value than any of the others (−23.0 to −33.0°), when measured in pyridine as the solvent. For the nitrophenylhydrazides, it was necessary to use 2-methoxyethanol as the solvent due to impaired visibility in pyridine. The *o*-nitro substituent caused a much greater specific rotation (−54.7°) than the *m*-nitro substituent (−34.4°). There is no general correlation between specific rotations and positions of substituents on the ring.

A general relationship does hold for melting points of any set of three arylhydrazides produced from three *N*-acylamino acids and a given arylhydrazine. The hippuric arylhydrazide always has the highest melting point, the *N*-(benzyloxycarbonyl)-L-alanine arylhydrazide is intermediate, and the *N*-(benzyloxycarbonyl)-glycine arylhydrazide is lowest, as shown in Table II.

The observed rates of formation of N^1-methyl-N^1-phenylhydrazides were very slow, particularly when the L- or racemic *N*-acylamino acid was used. The methyl group on the *N*-acylamino acid and on the hydrazino group appears to cause steric hindrance as the arylhydrazine approaches the acylated enzyme. The basicity of the NH_2 group is less sensitive to resonance or inductive properties of the ring substituents in the arylhydrazines[14] than in anilines[15] because of the insulating effect of the intervening NH. Similarly, steric effects are less apparent with arylhydrazines studied here than with anilines previously employed[16] in papain-catalyzed reactions.

Experimental Section

General Procedures.—A Rudolph high-precision polarimeter, purchased through funds from the Research Corp. of N. Y., was used for all polarimetric measurements. Water-jacketed polarimeter tubes, 1 or 2 dm, were used at a controlled temperature of 25°. With the exception of the nitro compounds, the solvent was Eastman Spectro Grade pyridine. Approximately 2% solutions (0.02 g/ml of solution) were made up in 5- or 10-ml volumetric flasks and were incubated in a constant temperature bath at 25°. For the nitro compounds, 2-methoxyethanol was used as the solvent. Nitrogen analyses were determined by the C. F. Geiger Microanalytical Laboratory, Ontario, Calif. All substituted phenylhydrazines were commercially available as their hydrochlorides or free bases. A few of these were also prepared by suitable modifications of established methods.[17-20]

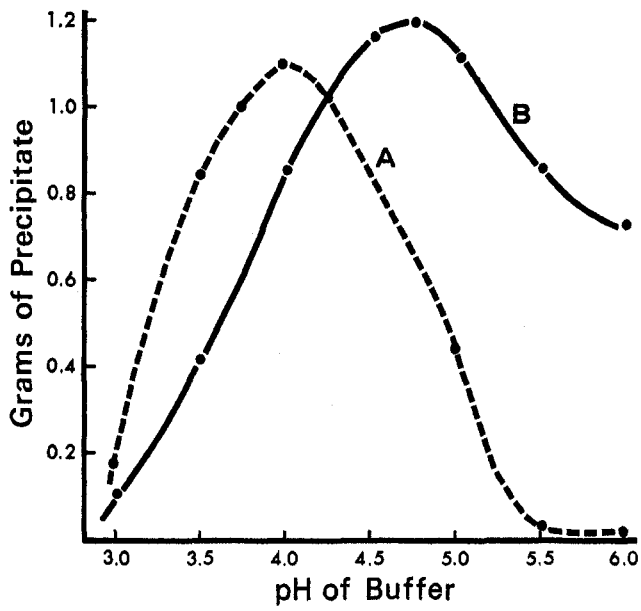

Figure 1.—Dependence of yield on pH for the formation of A, *N*-(benzyloxycarbonyl)glycine phenylhydrazide after 22 hr of incubation at 40°; B, *N*-(benzyloxycarbonyl)glycine N^1-methyl-N^1-phenylhydrazide after 24 hr of incubation at 40°. Solution constituents are as follows: 100 ml of 0.50 *M* buffer; 0.0100 mol of *N*-(benzyloxycarbonyl)glycine; 0.0100 mol of phenylhydrazine (A), or 0.0100 mol of N^1-methyl-N^1-phenylhydrazine (B); 0.250 g of papain; 0.500 g of L-cysteine·HCl·H_2O.

Dependence of Yield on pH.—The dependence of yield on pH was established for two reactions. Phenylhydrazine and N^1-methyl-N^1-phenylhydrazine were each subjected to reactions with *N*-(benzyloxycarbonyl)glycine in appropriate 0.50 *M* buffers at properly spaced pH values. A 100-ml portion of each buffer solution contained 0.0100 mol of each reactant, as well as 0.500 g of L-cysteine·HCl·H_2O and 0.250 g of activated papain. All solutions were filtered before addition of cysteine or papain. The latter two were ground in a mortar and then dissolved in a few milliliters of buffer before addition to the solution. The mortar was rinsed with the solution which was poured back into the reaction flask. Solutions were incubated for 22 hr in the case of phenylhydrazine, or for 24 hr for N^1-methyl-N^1-phenylhydrazine, at 40°. Precipitates were removed by suction filtration, dried in the atmosphere for 2 days, and weighed. Figure 1 shows the dependence of yield on pH for each reaction.

Preparation of Active Papin.—The papaya latex, imported from Ceylon, was donated by the Wallerstein Laboratories of N. Y. The procedure used for extraction and activation of papain was a

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TABLE II

FORMATION OF ARYLHYDRAZIDES BY PAPAIN-CATALYZED REACTIONS BETWEEN ARYLHYDRAZINES AND N-ACYLAMINO ACIDS AT pH 4.0 AND 40°

N-Acylamino acid[a]	Product[b]	Registry no.	Mp, °C	$[\alpha]^{25}D$, deg, in pyridine[c]	% N	
					Found	Calcd
HA	Hippuric phenylhydrazide (P)	6334-93-6	176–178			15.61
BzOC-G	*N*-(Benzyloxycarbonyl)glycine phenylhydrazide	21855-71-0	142–144		13.93	14.04
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine phenylhydrazide	28861-55-4	153–155	−31.9	13.21	13.41
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine phenylhydrazide		152–155	−24.4	Mmp, no depression[d]	
HA	Hippuric *o*-tolylhydrazide (oT)	10517-74-5	167–169		14.88	14.83
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *o*-tolylhydrazide	28861-57-6	66–68		12.67	12.84
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine *o*-tolylhydrazide	28861-58-7	165–166	−33.5	12.67	12.84
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine *o*-tolylhydrazide		165–167	−27.5	Mmp, no depression	
HA	Hippuric *m*-tolylhydrazide (mT)	10517-78-9	179–181		15.05	14.83
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *m*-tolylhydrazide	28861-60-1	124–125		13.70	13.41
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine *m*-tolylhydrazide	28861-61-2	143–144	−27.0	12.66	12.84
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine *m*-tolylhydrazide		143–145	−23.3	Mmp, no depression	
HA	Hippuric *p*-tolylhydrazide (pT)	10517-82-5	175–177		15.12	14.83
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *p*-tolylhydrazide	28861-63-4	133–135		13.75	13.41
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine *p*-tolylhydrazide	28861-64-5	160–162	−29.0	13.10	12.8
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine *p*-tolylhydrazide		162–165	−27.8	Mmp, no depression	
HA	Hippuric *o*-methoxyphenylhydrazide (oM)	28861-65-6	148–149		13.98	14.04
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *o*-methoxyphenylhydrazide	28861-66-7	76–78		12.50	12.76
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine *o*-methoxyphenylhydrazide	28861-67-8	159–160	−24.7	12.50	12.76
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine *o*-methoxyphenylhydrazide		158–160	−22.6	Mmp, no depression	
HA	Hippuric *p*-methoxyphenylhydrazide (pM)	28861-68-9	179–180		14.62	14.69
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *p*-methoxyphenylhydrazide	28861-69-0	140–142		12.69	12.76
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine *p*-methoxyphenylhydrazide	28861-70-3	162–165	−29.4	11.94	12.24
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine *p*-methoxyphenylhydrazide		160–162	−22.4	Mmp, no depression	
HA	Hippuric *o*-fluorophenylhydrazide (oF)	28861-71-4	179–181		14.30	14.63
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *o*-fluorophenylhydrazide	28861-72-5	94–95		13.49	13.25
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine *o*-fluorophenylhydrazide	28861-73-6	151–153	−23.05	13.01	12.77
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine *o*-fluorophenylhydrazide		156–158	−23.00	Mmp, no depression	
HA	Hippuric *m*-fluorophenylhydrazide (mF)	28861-74-7	177–179		14.79	14.63
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *m*-fluorophenylhydrazide	28861-75-8	132–133		13.12	13.25
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine *m*-fluorophenylhydrazide	28861-76-9	156–158	−27.5	12.43	12.77
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine *m*-fluorophenylhydrazide		156–158	−26.9	Mmp, no depression	
HA	Hippuric *p*-fluorphenylhydrazide (pF)	28861-77-0	192–194		14.50	14.63
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *p*-fluorophenylhydrazide	28860-82-4	142–144		12.95	13.25
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine *p*-fluorophenylhydrazide	28860-83-5	173–174	−24.8	12.88	12.77

TABLE I (*Continued*)

N-Acylamino acid[a]	Product[b]	Registry no.	Mp, °C	$[\alpha]^{25}D$, deg, in pyridine[c]	% N Found	% N Calcd
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine *p*-fluorophenylhydrazide		174–176	−24.2	Mmp, no depression	
HA	Hippuric *o*-nitrophenylhydrazide (oN)	28860-84-6	206–208		17.60	17.83
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *o*-nitrophenylhydrazide	28860-85-7	163–165		16.36	16.28
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine[c] *o*-nitrophenylhydrazide	28860-86-8	190–191	−54.7	15.90	15.74
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine[c] *o*-nitrophenylhydrazide		188–190	−53.8	Mmp, no depression	
HA	Hippuric *m*-nitrophenylhydrazide (mM)	28860-87-9	182–184		18.08	17.83
BzOC-G	*N*-(Benzyloxycarbonyl)glycine *m*-nitrophenylhydrazide	28860-88-0	149–150		16.55	16.28
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine[c] *m*-nitrophenylhydrazide	28860-89-1	154–156	−34.4	15.44	15.74
BzOC-DL-A	N-(Benzyloxycarbonyl)alanine[c] *m*-nitrophenylhydrazide		154–156	−34.0	Mmp, no depression	
HA	Hippuric N^1-methyl-N^1-phenylhydrazide (MP)[e]	28860-90-4	161–162		14.47	14.83
BzOC-G	*N*-(Benzyloxycarbonyl)glycine N^1-methyl-N^1-phenylhydrazide	28860-91-5	120–122		13.53	13.41
BzOC-L-A	*N*-(Benzyloxycarbonyl)-L-alanine N^1-methyl-N^1-phenylhydrazide	28860-92-6	152–154	−16.4	12.80	12.84
BzOC-DL-A	*N*-(Benzyloxycarbonyl)alanine N^1-methyl-N^1-phenylhydrazide		150–153	−10.6	Mmp, no depression	

[a] HA = hippuric acid; BzOC-G = *N*-(benzyloxycarbonyl)glycine; BzOC-L-A = *N*-(benzyloxycarbonyl)-L-alanine; BzOC-DL-A = *N*-(benzyloxycarbonyl)-DL-alanine. [b] Abbreviations in parentheses are for the arylhydrazine reactant, used in giving weights of products from reactants in the Experimental Section. [c] Pyridine was used for all solutes except the nitro compounds, in which case 2-methoxyethanol was the solvent. [d] This designates that a mixture of the products from the pure L-amido and the DL-amido acid gave no depression of melting point so that no nitrogen analysis was necessary. Mmp = mixture melting point. [e] The pH for the N^1-methyl-N^1-phenylhydrazides was 4.6 rather than 4.0.

modification of the method used by Bennett and Niemann.[21] Two 100-g samples of the latex were ground in a mortar. Each portion was poured into a 500-ml suction flask that contained 100 ml of distilled water. Each flask was placed in an ice bath and each mixture was stirred for 4 hr with mechanical stirrers. This was followed by two centrifugations of each solution at 2000 rpm for 20 min, each time, to remove undissolved latex. The liquid solutions were decanted into two 500-ml suction flasks in ice baths, in separate dewar flasks. Hydrogen sulfide was passed slowly and simultaneously into each solution for 12 hr. At the end of this period, 5 g of Celite filter aid was added to each flask and each solution was shaken for several minutes. Two centrifugations of each mixture at 2000 rpm, followed by decantation each time, produced a slightly turbid solution which was decanted and suction filtered to remove traces of filter aid. Sufficient methanol was added to each solution to produce a 70% by volume methanolic solution. Precipitates were removed by centrifugation at 2000 rpm for 20 min. The creamed-colored papain paste was scraped into a deep glass dish and dried over P_2O_5 for 5 days. It was necessary to pour off methanol at the end of 24-hr periods and to replenish the P_2O_5. A light tan, brittle solid resulted amounting to about 46 g. It was stored in a refrigerator at 5° in two dark green bottles with screw caps. Its activity was excellent as shown by a trial reaction with phenylhydrazine and *N*-(benzyloxycarbonyl)glycine.

Procedure for Papain-Catalyzed Reactions with Hippuric Acid, *N*-(Benzyloxycarbonyl)glycine, and *N*-(Benzyloxycarbonyl)-L-alanine.—For phenylhydrazine and ring-substituted phenylhydrazines, reactions were run at a pH of 4.0, which was the optimum shown for phenylhydrazine and *N*-(benzyloxycarbonyl)-glycine. A total of 100 ml of 0.50 *M* formic acid buffer was used. Then, 0.0100 mol of the phenylhydrazine or its salt, 0.0100 mol of the *N*-acylamino acid, and 0.250 g of L-cysteine·HCl·H_2O were ground and shaken with 95 ml of the buffer for 25–30 min, followed by suction filtration. The pH was adjusted to 4.0, if necessary. Buffer (5 ml) was used to triturate 0.300 g of papain until it dissolved. It was rinsed with reactant solution into the reaction flask. Incubation was carried out at 40°. Solid was removed by suction filtration at the end of 24 and 28 hr and then dried in the atmosphere for about 2 days and weighed.

Procedure for Papain-Catalyzed Reactions with *N*-(Benzyloxycarbonyl)-DL-alanine.—For these reactions, 0.00600 mol of the phenylhydrazine or its salt was used with 0.0120 mol of *N*-(benzyloxycarbonyl)-DL-alanine. Otherwise the procedure was the same with 100 ml of formic acid buffer, pH 4.0.

Procedure for Papain-Catalyzed Reactions with N^1-Methyl-N^1-phenylhydrazine.—Since the pH optimum was 4.75 for the reaction with *N*-(benzyloxycarbonyl)glycine, a 0.50 *M* acetic acid buffer at pH 4.6 was found to be convenient for these reactions. For hippuric acid, 0.0100 mol of substituted phenylhydrazine and amido acid were employed. For *N*-(benzyloxycarbonyl)-L-alanine and *N*-(benzoyloxycarbonyl)-DL-alanine, so little reaction product was given by the usual method that the amount of solution was increased to 250 ml, while 1.000 g of papain was used and about 0.600 g of L-cysteine·HCl·H_2O. About 0.0250 mol of the *N*-acyl-L-amino amido acid was used, and about the same quantity of the *N*-acyl-DL-amino acid was also used. Several variations were tried and products were accumulated in each case. Only about 0.25 g of product was obtained at the end of 48 hr of incubation at pH 4.6 and 40°.

Hippuric *m*-Tolylhydrazide and Hippuric *p*-Fluorophenylhydrazide.—It was necessary to run several trial experiments to obtain sufficient amounts of these products for purification, for nitrogen analyses, and determination of melting points. In general, about 200 ml of solutions were made up with nearly 0.02 mol of the substituted phenylhydrazide. The same amount of hippuric acid was used with 0.500 g of L-cysteine·HCl·H_2O and 1 g of papain. After 48 hr, between 0.15 and 0.20 g of product was obtained when the usual drying procedure was used. In all trial runs with *p*-nitrophenylhydrazine and any of the *N*-acylamino acids, no product was obtained. This was due to the extreme insolubility of *p*-nitrophenylhydrazine in the buffer at pH 4.0.

Purification Procedures.—When the reaction mixtures were filtered before incubation, clear-cut precipitates were usually obtained by filtration of the product mixtures. After suction filtration and drying the solids in the atmosphere for 48 hr, the solids were stirred with several portions of hot water and removed

(21) E. L. Bennett and C. Niemann, *J. Amer. Chem. Soc.*, **72**, 1798 (1950).

by filtration. This dissolved soluble impurities. They were again dried in the atmosphere and then dissolved in cold methanol and treated with carbon black in the cold for about 10 min. Filtration was repeated four times under suction. Each time meticulously cleaned funnels and flasks were used to ensure complete removal of carbon and insoluble impurities, as well as those adsorbed by the carbon. The clear solutions were poured into petri dishes and allowed to evaporate under the hood. Solids were removed and dried over phosphorus pentoxide in a vacuum desiccator. All nitrophenylhydrazides were deep yellow in color. Other solids took on slight coloration if exposed to the atmosphere too long. This seems to be due to the susceptibility of these substituted hydrazines to slight atmospheric oxidation. Further purification was unnecessary for obtaining correct nitrogen analyses, nor did further purification improve the melting points or optical rotations.

Melting Points.—All melting points were determined by means of a Fisher-Johns melting point apparatus. Corrections were made by the use of several compounds of proper purity and known melting points. A curve was plotted for their measured melting points along with a straight line curve for true melting points, in the usual manner. Substances used were *p*-dichlorobenzene, *m*-dinitrobenzene, benzoic acid, salicylic acid, hippuric acid, and anthracene.

Yields of Arylhydrazides from Listed Reactants.—The reactants and yields for periods of incubation of 0–24 hr (24–48 hr in parentheses) follow (abbreviations for reactants are indicated in Table II; details of the experiments are provided elsewhere in the Experimental Section): HA + P, 0.853 g (0.246 g); BzOC-G + P, 1.370 g (0.223 g); BzOC-L-A + P, 1.576 g (0.0414 g); BzOC-DL-A + P, 1.525 g (0.0203 g); HA + oT, 0.396 g (0.324 g); BzOC-G + oT, 0.821 g (0.314 g); BzOC-L-A + oT, 1.557 g (0.0782 g); BzOC-DL-A + oT, 1.032 g (0.0999 g); HA + mT (see Experimental Section); BzOC-G + mT, 0.619 g (0.105 g); BzOC-L-A + mT, 1.718 g (0.0356 g); BzOC-DL-A + mT, 1.533 g (0.0815 g); HA + pT, 0.654 g (0.223 g); BzOC-G + pT, 1.256 g (0.247 g); BzOC-L-A + pT, 1.360 g (0.0042 g); BzOC-DL-A + pT, 0.378 g (0.0261 g); HA + oM, 0.674 g (0.254 g); BzOC-G + oM, 0.888 g (0.201 g); BzOC-L-A + oM, 2.067 g (0.0419 g); BzOC-DL-A + oM, 0.864 g (0.0086 g); HA + pM, 0.0523 g (0.0202 g); BzOC-G + pM, 0.209 g (0.0185 g); BzOC-L-A + pM, 0.271 g (0.0312 g); BzOC-DL-A + pM, 0.188 g (0.0467 g); HA + oF, 0.925 g (0.207 g); BzOC-G + oF, 1.495 g (0.160 g); BzOC-L-A + oT, 1.154 g (0.0743 g); BzOC-DL-A + oF, 1.353 g (0.0635 g); HA + mF, 0.363 g (0.143 g); BzOC-G + mF, 0.647 g (0.0769 g); BzOC-L-A + mF, 0.173 g (0.0452 g); BzOC-DL-A + mF, 0.361 g (0.0700 g); HA + pF (see Experimental Section); BzOC-G + pF, 0.163 g (0.0475 g); BzOC-L-A + pF, 0.256 g (0.163 g); HA + oN, 0.132 g (0.000 g); BzOC-G + oN, 0.197 g (0.000 g); BzOC-L-A + oN, 0.205 g (0.000 g); BzOC-DL-A + oN, 0.232 g (0.000 g); HA + mN, 0.293 g (0.0717 g); BzOC-G + mN, 0.518 g (0.0850 g); BzOC-L-A + mN, 0.608 g (0.0558 g); BzOC-DL-A + mN, 0.402 g (0.0658 g); HA + MP, 0.000 g (0.203 g); BzOC-G + MP, 0.206 g (0.198 g); BzOC-L-A MP and BzOC-DL-A + MP (see Experimental Section).

Registry No.—Phenylhydrazine, 100-63-0; *N*-(benzyloxycarbonyl)-DL-alanine, 4132-86-9.

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Studies on Reactions of Isoprenoids. XIII.[1] The 1,4-Cycloaddition Reactions of Alloocimene with Various Dienophiles

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1,4-Cycloaddition reactions of alloocimene, an isomeric mixture of **1a** and **1b**, with several acetylenic, olefinic, and heterodienophiles were investigated. With cyanoacetylene, alloocimene afforded a 1:1 adduct **2a** and *a 1:2 adduct* **3**; **3** is regarded as a 1,4 cycloadduct of a 1:1 ene product from **1b** to cyanoacetylene. With dimethyl acetylenedicarboxylate, tetracyanoethylene, *p*-benzoquinone, and 4-phenyl-1,2,4-triazoline-3,5-dione, both **1a** and **1b** gave the corresponding 1,4 adducts, but, with nitrosobenzene, only **1a** reacted to afford two isomeric adducts, **11a** and **12a**, having a different orientation. All the 1,4-cycloaddition reactions proceeded primarily by reaction of **1a** rather than **1b**.

Although 1,4-cycloaddition reactions of alloocimene, 2,6-dimethyl-2,4,6-octatriene, with maleic anhydride[2] and dialkyl azodicarboxylate[3] have been studied extensively, those with other dienophiles have apparently not yet been investigated. As an extension of our study on the 1,4-cycloaddition reactions of myrcene,[1,4] this paper deals with the reactions of alloocimene with several acetylenic, olefinic, and heterodienophiles of unsymmetrical structure such as cyanoacetylene and nitrosobenzene. Regiospecific properties of the cycloadditions are discussed.

(1) Part XII of this series: T. Sasaki, S. Eguchi, T. Ishii, and H. Yamada, *J. Org. Chem.*, **35**, 4273 (1970).

(2) (a) J. E. Milks and J. E. Lancaster, *ibid.*, **30**, 888 (1965); (b) E. K. von Gustorf and J. Leitich, *Tetrahedron Lett.*, 4689 (1968), and references cited therein.

(3) E. K. von Gustorf, *ibid.*, 4693 (1968).

(4) T. Sasaki, S. Eguchi, and T. Ishii, *J. Org. Chem.*, **34**, 3749 (1969).

Results and Discussion

Reaction Conditions.—The alloocimene used in all the reactions was an isomeric mixture of **1a** and **1b**[5] in a 57:43 ratio, since the interconversion of **1a** and **1b** by dienophiles has been reported[2b] and since the separation of **1a** and **1b** requires considerable effort. Reaction conditions, product distribution, and yields as well as the isomer ratios of the recovered alloocimene and the products are summarized in Table I. Reaction temperatures were chosen so as to give the adducts under as mild conditions as possible. The polymerization of alloocimene was not serious under the employed conditions and no polymerization inhibitor was used.

In reactions with acetylenic dienophiles, such as

(5) For nomenclature of the two geometrical isomers, **1a** and **1b**, see K. J. Crowley, *ibid.*, **33**, 3679 (1968).