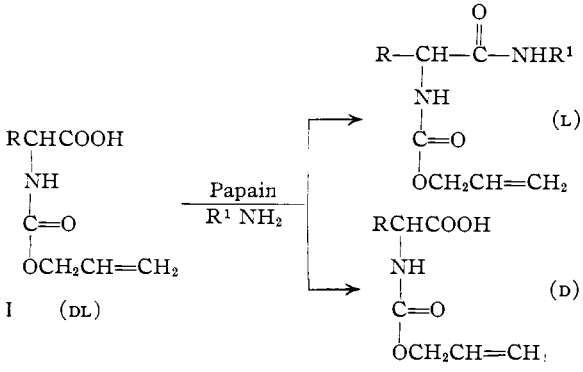
[CONTRIBUTION FROM THE FULMER CHEMICAL LABORATORY, THE STATE COLLEGE OF WASHINGTON]

Amino Acid Derivatives. II. Enzymatic Synthesis of Phenylhydrazides of Carboallyloxyamino Acids[1,2]

BY H. BAYARD MILNE AND CARL M. STEVENS

In a previous paper,[3] the preparation of carboallyloxy derivatives (I) of a series of α-amino acids has been reported, together with methods for subsequent cleavage of the carboallyloxy group. The success of these studies suggested the use of the carboallyloxy derivatives for the enzymatic resolution of amino acids. The original studies of Bergmann and co-workers,[4,5] and subsequent work of other investigators[6,7,8] demonstrated a high degree of stereochemical specificity for enzymatic syntheses involving papain. It seemed probable, therefore, that the reaction with the carboallyloxyamino acids would proceed according to the scheme



thus providing a convenient method of resolution of the racemic derivative. In the present study, phenylhydrazine was selected as the amine because of the possibility of subsequent removal of the phenylhydrazine grouping by mild oxidation.[9]

Incubation of the series of carboallyloxy amino acids with phenylhydrazine in the presence of papain yielded in every case crystalline phenylhydrazides which could be obtained in analytically pure form. When the starting material was a non-asymmetric compound or the derivative of an L-amino acid, the corresponding hydrazides showed constant melting point and, if optically active, constant rotation (Table I).

On the other hand, in certain instances when the starting compound was the derivative of a DL-amino acid, crops of hydrazide collected at different times showed different melting points and optical rotations, despite the fact that all crops appeared to be analytically pure. This is illustrated by a comparison of successive fractions of phenylhydrazide obtained from the reaction of the N-carboallyloxy derivatives of L-leucine and DL-leucine (Table II).

TABLE I

CARBOALLYLOXYAMINO ACID PHENYLHYDRAZIDES

Amino acid	M. p. of phenylhydrazide, °C.	Solvent for recrystn.	$[\alpha]^{25}D$ (chloroform)	Formula	Nitrogen anal., % Calcd.	Found
Glycine	114–115	Toluene	.....	$C_{12}H_{15}O_3N_3$	16.8	16.3
L-Leucine	111–112	Toluene	−68.5°	$C_{18}H_{23}O_3N_3$	13.8	13.8
L-Lysine[a]	132–133	Toluene	−30.4°	$C_{20}H_{28}O_5N_4$	13.9	13.7

[a] N,N-Dicarboallyloxy-L-lysine was used in this reaction.

TABLE II

ENZYMATIC SYNTHESIS OF PHENYLHYDRAZIDES

Substrate	Formation of phenylhydrazide, hr.	Yield of phenylhydrazide, g.	Properties of phenylhydrazide M. p., °C.	$[\alpha]^{25}D$ ($CHCl_3$)
N-Carboallyloxy-L-leucine	0–18	14.1	112–113	−68.0[a]
N-Carboallyloxy-L-leucine	18–48	3.4	112–113	−68.5
N-Carboallyloxy-L-leucine	48–144	2.0	112–113	−68.3
N-Carboallyloxy-DL-leucine	0–18	15.3	110–112	−63.7
N-Carboallyloxy-DL-leucine	18–48	4.9	110–120	−55.7
N-Carboallyloxy-DL-leucine	48–72	1.1	135–148	−30.2
N-Carboallyloxy-DL-leucine	72–144	2.3	150–153	−14.2[b]

[a] Analysis: N, 13.9. [b] Analysis: N, 13.8.

The results suggest that the enzymatic synthesis is not completely asymmetric in this instance, but that the D-amino acid derivative reacts to a small but appreciable extent. This was conclusively demonstrated in a subsequent experiment in which N-carboallyloxy-D-leucine was incubated with phenylhydrazine in the presence of papain. Pure N-carboallyloxy-D-leucine phenylhydrazide (m. p. 111–112°) was obtained.

After these experiments were completed, Bennett and Niemann[10] reported evidence of an enzymatic synthesis involving N-carbobenzoxy-D-*o*-fluorophenylalanine, and more recently[11] the same investigators have extended their study to other derivatives of phenylalanine. The data obtained in our studies provide an example of lack of absolute stereochemical specificity in enzymatic syntheses in a different series of compounds. The data also demonstrate enzymatic synthesis when

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(2) Presented at the 112th meeting of the American Chemical Society, New York, N. Y., September 16, 1947.

(3) Stevens and Watanabe, THIS JOURNAL, **72**, 725 (1950).

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

(5) Fruton, Irving and Bergmann, *ibid.*, **133**, 703 (1940).

(6) Niemann and Nichols, *ibid.*, **143**, 191 (1942).

(7) Dekker and Fruton, *ibid.*, **173**, 471 (1948).

(8) Hanson and Smith, *ibid.*, **179**, 815 (1949).

(9) *Cf.* Hann and Hudson, THIS JOURNAL, **56**, 957 (1934).

(10) Bennett and Niemann, *ibid.*, **70**, 2610 (1948).

(11) Bennett and Niemann, Abstracts of Papers, 115th Meeting, American Chemical Society, 32C (1949).

the starting material is the pure derivative of D-configuration.

Experimental

Carboallyloxyamino Acids.—The carboallyloxy amino acids were prepared as described in a previous article.[8]

Papain.—Commercial papain (Merck) was purified by the general procedure of Grassmann,[12] and Bergmann and Fraenkel-Conrat.[4] After two successive treatments with hydrogen sulfide, followed by precipitation with methanol, the preparation was lyophilized, yielding a light powder.

Enzymatic Syntheses.—The carboallyloxyamino acid phenylhydrazides were prepared by incubating carboallyloxyamino acids with phenylhydrazine in a buffered solution of papain and cysteine hydrochloride at 40° according to the method of Bergmann and Fraenkel-Conrat.[4] Certain of the results are listed in Table I.

Comparison of Enzymatic Synthesis from Derivatives of L-Leucine and DL-Leucine.—Two parallel runs were made. In one experiment, 0.2 mole (43 g.) of N-carboallyloxy-DL-leucine was incubated with 10 ml. of phenylhydrazine, 4 g. of L-cysteine hydrochloride, and 2 g. of papain in 1 l. of solution buffered with acetic acid–sodium acetate to *p*H 4.8. In the other experiment, 0.1 mole (21.5 g.) of N-carboallyloxy-L-leucine was incubated with 10 ml. of phenylhydrazine, 2 g. of L-cysteine hydrochloride, and 1 g. of papain in 500 ml. of solution, buffered with acetic acid–sodium acetate at *p*H 4.8. At intervals, the solid product was collected, and the filtrates incubated further. The melting point and specific rotation was taken for each sample of precipitate. The results are shown in Table II.

N-Carboallyloxy-D-leucine.—After the mixture of N-carboallyloxy-DL-leucine, papain and phenylhydrazine described above had incubated for 144 hours, 23.6 g. of N-carboallyloxyleucine of predominantly the L-form had been removed from the solution. The solution containing unreacted N-carboallyloxy-D-leucine was evaporated to dryness. The resulting solid was dissolved in 100 ml. of 6 *N* hydrochloric acid and heated under reflux for three hours. This solution was then evaporated to dryness, the residue dissolved in 50 ml. of water, and concd. ammonium hydroxide added to *p*H 6. After cooling, 0.8 g. of crystals was collected. When recrystallized from alcohol–water, the yield of D-leucine was 0.55 g., $[\alpha]^{20}D$ −14.8° (3.6% in 20% hydrochloric acid). The D-leucine was converted to N-carboallyloxy-D-leucine by the usual method.

N-Carboallyloxy-D-leucine Phenylhydrazide.—A solution consisting of 0.2 g. of N-carboallyloxy-D-leucine, 0.067 g. of L-cysteine hydrochloride, 0.05 g. of purified papain, and 0.5 ml. of phenylhydrazine in 15 ml. of solution buffered to *p*H 4.8 was incubated at 40°. After twelve days the insoluble product was collected, yielding 0.177 g. of N-carboallyloxy-D-leucine phenylhydrazide; m. p. 110°. Recrystallized from toluene, it melted at 111–112°. When mixed with an equal amount of N-carboallyloxy-L-leucine phenylhydrazide, the melting point was 153–154°.

(12) Grassmann, *Biochem. Z.*, **279**, 131 (1935).

Summary

From the treatment of a series of N-carboallyloxy-derivatives of α-amino acids with phenylhydrazine in the presence of papain, the crystalline phenyl hydrazides have been obtained in every case. While the carboallyloxy derivatives of L-amino acids yield the corresponding L-phenylhydrazides the derivatives of DL-amino acids do not invariably yield the optically pure L-phenylhydrazide. Under the conditions of the experiments, the D-phenylhydrazide is simultaneously formed to an appreciable though much smaller extent.

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Reactions of Vanillin and its Derived Compounds. IX.[1] Some New Esters of Syringic and Protocatechuic Acids[2]

BY IRWIN A. PEARL AND DONALD L. BEYER

The possible utilization of the lignin and/or the waste liquors from the sulfite pulping of coniferous woods by oxidation to vanillin and transformation of the resulting vanillin to vanillic acid has been reviewed[3] and the direct oxidation of lignosulfonates to vanillic acid has been reported recently.[4] Ester of vanillic acid and of relateds acids derived from vanillin have been prepared and tested for their toxicity toward representative microörganisms and their ultraviolet absorption properties.[5]

Oxidations of lignins and sulfite waste liquors derived from the pulping of hardwoods by processes similar to those employed with softwood lignins yield considerable amounts of syringaldehyde and syringic acid in addition to vanillin and vanillic acid. This fact, together with the well-known fact that more and more hardwoods are being processed by our sulfite pulp mills, led to an investigation of the esters of syringic acid.

Syringic acid for this study was prepared by treatment of trimethylgallic acid with concentrated sulfuric acid or with fuming sulfuric acid by the method of Bogert and Coyne[6] and Bogert and Ehrlich,[7] respectively. When employing the concentrated sulfuric acid method, we found it impossible to obtain syringic acid free from trimethylgallic acid unless the trimethylgallic acid was recrystallized before the sulfuric acid treatment. Such recrystallization before treatment obviated the necessity for purification of the syringic acid which could be employed as such for the esterifi-

(1) For paper VIII of this series, see THIS JOURNAL, **71**, 2331 (1949).

(2) This paper represents a portion of the results obtained in the research program sponsored by the Sulphite Pulp Manufacturers' Research League and conducted for the League by The Institute of Paper Chemistry. Acknowledgment is made by the Institute for permission on the part of the League to publish these results.

(3) Pearl, *Chem. Eng. News*, **26**, 2952 (1948).

(4) Pearl, THIS JOURNAL, **71**, 2196 (1949).

(5) (a) Pearl and McCoy, *ibid.*, **69**, 3071 (1947); (b) Pearl and Beyer, *ibid.*, **71**, 1066 (1949); (c) Pearl, *ibid.*, **71**, 2331 (1949).

(6) Bogert and Coyne, *ibid.*, **51**, 571 (1929).

(7) Bogert and Ehrlich, *ibid.*, **41**, 799 (1919).