The Preparation and Reactions of N-Carboxyphenylhydrazidoamino Acids[1]

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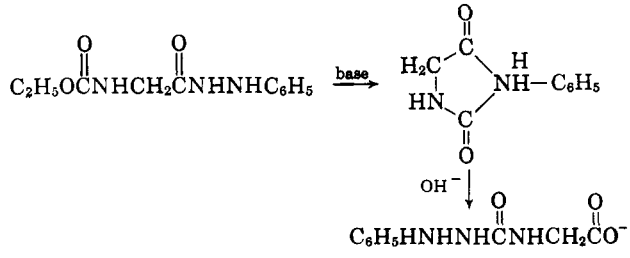
N-Carboxyphenylhydrazido derivatives of glycine, DL-alanine, DL-phenylalanine, DL-leucine, and DL-tyrosine have been prepared by refluxing carboalkoxyamino acid phenylhydrazides with potassium hydroxide in ethanol. The 5-substituted 3-anilinohydantoins were isolated as intermediates in the above reactions. The N-carboxyphenylhydrazidoamino acids were oxidized with potassium permanganate to give N-phenylazocarbonyl derivatives of the amino acids. The N-carboxyphenylhydrazido-DL-amino acids were used as substrates in a papain-catalyzed reaction with phenylhydrazine to give N-carboxyphenylhydrazido-L-amino acid phenylhydrazides.

In a recent report[2] base-catalyzed cyclizations of S-benzylthiocarbonylglycine phenylhydrazide, carboethoxyglycine phenylhydrazide, and N-carboxyphenylhydrazidoglycine ethyl ester to 3-anilinohydantoin were reported. 3-Anilinohydantoin, when hydrolyzed, gave N-carboxyphenylhydrazidoglycine. A reaction was also reported[2] in which carboethoxyglycine phenylhydrazide reacted in alcoholic potassium hydroxide solution to give the potassium salt of N-carboxyphenylhydrazidoglycine. It was postulated that this reaction proceeded through the 3-anilinohydantoin intermediate.

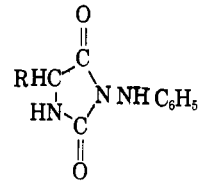


On the basis of more experiments, it appears that this is a general reaction. Several carbobenzoxy-, carboethoxy-, and carboallyloxyamino acid phenylhydrazides have been refluxed with 0.1 *N* potassium hydroxide in absolute ethanol to give N-carboxyphenylhydrazido derivatives of glycine, DL-alanine, DL-leucine, DL-phenylalanine, and DL-tyrosine (Table I).

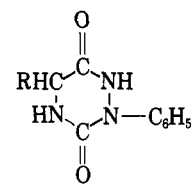
The structures of the N-carboxyphenylhydrazidoamino acids were confirmed by their infrared and ultraviolet spectra and by oxidizing them to N-phenylazocarbonyl derivatives. The infrared spectra of the N-carboxyphenylhydrazidoamino acids all showed a single NH peak at 3.08 μ and an amide II absorption at 6.45 μ characteristic of secondary amides.[3] Their ultraviolet spectra had maxima at 233 and 282 mμ, characteristic of 2-acyl-1-phenylhydrazines.[4]

By reducing the concentration of base used in the above reaction, it was possible to isolate the intermediate 3-anilinohydantoins. When the carbobenzoxy-, carboethoxy-, and carboallyloxyamino acid phenylhydrazides were refluxed in 0.01 *N* potassium hydroxide in absolute ethanol from 1–2 hr., the 5-substituted 3-anilinohydantoins were isolated (Table II). The yields ranged from 22.4 in the case of 3-anilinohydantoin to 92.5% in the case of 5-benzyl-3-anilinohydantoin.[5]

That these compounds have the 3-anilinohydantoin structure (I) and not the 2-phenyl-3,6-dioxohexahydro-1,2,4-triazine structure (II) was indicated by infrared



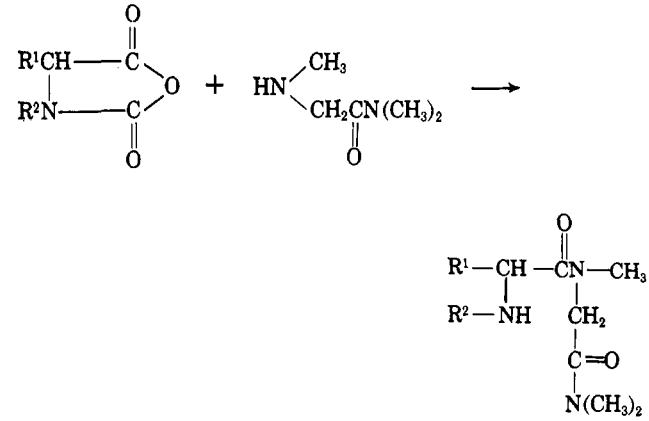
I



II

and ultraviolet spectra. The infrared spectra all had carbonyl stretching frequencies at 5.62 and at 5.74 μ, and the ultraviolet spectra showed maxima at 228 and 278 mμ, characteristic of 3-anilinohydantoins.[2]

In order to find an explanation for the varying yields of 5-substituted 3-anilinohydantoins, the kinetics of the base-catalyzed hydrolyses of 3-anilinohydantoin, 5-methyl-3-anilinohydantoin, and 5-isobutyl-3-anilinohydantoin were determined. Fairly constant values for second-order rate constants were obtained (Table III). The rate was dependent on the size of the substituent in the 5-position, with 3-anilinohydantoin reacting at almost twice the rate of 5-isobutyl-3-anilinohydantoin. That the rates of formation of the 3-anilinohydantoins are much faster than their rates of hydrolysis is shown by the fact that the $T_{1/2}$ for the rearrangement of N-carbobenzoxy-L-leucine phenylhydrazide to N-carboxyphenylhydrazido-DL-leucine ($T_{1/2}$ = 19.25 min.) was about the same as the $T_{1/2}$ for the hydrolysis of 5-isobutyl-3-anilinohydantoin ($T_{1/2}$ = 19.18 min.) under



(1) This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

(2) H. B. Milne and D. W. Fish, *J. Org. Chem.*, **27**, 3177 (1962).

(3) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 206–209.

(4) N. A. Valyashko and I. T. Depeshko, *Zh. Obshch. Khim.*, **20**, 1667 (1950).

(5) Because of the similarities of the structures of these hydantoins to hydantoins which have shown anticonvulsant activity, they were tested by the Sterling-Winthrop Research Institute for hexobarbital potentiation, anticonvulsant activity, and effect on psychomotor activity. The activity was of very low order in each case.

TABLE I

N-CARBOXYPHENYLHYDRAZIDOAMINO ACIDS

N-Carboxyphenylhydrazidoamino acid	Reaction time, min.	Yield,[a] %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Glycine[b]	60	70	199–200	$C_9H_{11}N_3O_3$						
DL-Alanine	90	68	204–205	$C_{10}H_{13}N_3O_3$	53.80	54.03	5.87	6.00	18.83	19.02
DL-Leucine	180	68	169–170	$C_{13}H_{19}N_3O_3$	58.85	59.07	7.22	7.34	15.84	15.77
DL-Phenylalanine	90	65	188–190	$C_{16}H_{17}N_3O_3$	64.20	64.42	5.72	5.88	14.04	14.28
DL-Tyrosine	150	70	197–198	$C_{16}H_{17}N_3O_4$	60.94	60.74	5.43	5.48	13.33	13.07

[a] After one recrystallization from ethanol–water. [b] A mixture (m.p. 198–199°) showed no depression, and the infrared spectrum was identical with that of an authentic sample of N-carboxyphenylhydrazidoglycine previously prepared in this laboratory.[3]

TABLE II

PROPERTIES AND ANALYSES OF 5-SUBSTITUTED 3-ANILINOHYDANTOINS

5-Substituent	Reaction time, min.	Yield,[a] %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
H[b]	60	22.4[c]	163–165	$C_9H_9N_3O_2$						
CH_3	90	53.5	184–185	$C_{10}H_{11}N_3O_2$	58.53	58.50	5.41	5.47	20.48	20.72
$(CH_3)_2CHCH_2$	90	89.5	148–149	$C_{13}H_{17}N_3O_2$	63.14	63.03	6.93	6.84	16.99	17.08
$C_6H_5CH_2$	90	92.5	174–175	$C_{16}H_{15}N_3O_2$	68.31	68.19	5.37	5.50	14.94	14.69
$HOC_6H_4CH_2$	120	34.0	254–256	$C_{16}H_{15}N_3O_3$	64.63	64.37	5.09	4.98	14.13	14.02

[a] After one recrystallization from ethanol–water. [b] A mixture (m.p. 161–163°) showed no depression, and the infrared spectrum was identical with that of an authentic sample of 3-anilinohydantoin previously prepared in this laboratory. [c] Recrystallized from methanol–ether.

TABLE III

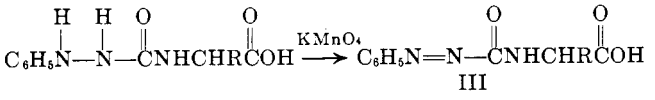
KINETICS OF THE HYDROLYSIS OF 5-SUBSTITUTED 3-ANILINOHYDANTOINS

3-Anilinohydantoin: Time, min.	Acid (0.1 N), ml.	k_2, min.$^{-1}$ mole^{-1} l.	5-Methyl-3-anilinohydantoin: Time, min.	Acid (0.1 N), ml.	k_2, min.$^{-1}$ mole^{-1} l.	5-Isobutyl-3-anilinohydantoin: Time, min.	Acid (0.1 N), ml.	k_2, min.$^{-1}$ mole^{-1} l.
0.5	9.56	0.92	0.6	9.56	0.78	1.5	9.21	0.57
2.0	8.46	0.91	1.0	9.18	0.89	3.0	8.85	0.43
3.5	7.52	0.94	3.0	8.10	0.78	6.0	7.25	0.63
6.0	6.54	0.88	5.0	7.06	0.83	9.0	6.60	0.57
8.0	5.90	0.89	7.0	6.52	0.76	14.0	5.82	0.51
11.5	4.80	0.94	9.5	5.52	0.85	19.0	4.93	0.55
14.8	4.14	0.95	11.0	5.18	0.85	26.0	4.48	0.47
17.6	3.72	0.96	13.0	4.86	0.81	33.0	3.82	0.49
25.0	2.81	1.03	16.0	4.30	0.83	40.0	3.40	0.47
38.0	2.00	1.05	22.0	3.24	0.95	50.0	2.82	0.51
	Av.	0.95		Av.	0.83		Av.	0.52

the same conditions. These observations are in agreement with the results reported by Ballard and Bamford,[6] who found that, in the second-order reaction of N-carboxyamino acid anhydrides with glycine trimethylamide, the rates depended upon the size of R^1. The rate was slower when $R^1 = CH_3$ than when $R^1 = H$. Bender[7] attributed this difference in rates to the steric repulsion of large groups in the transition state.

When optically active carboalkoxy-L-amino acids were used in the preparation of the 5-substituted 3-anilinohydantoins and the N-carboxyphenylhydrazidoamino acids, the resulting 5-substituted 3-anilinohydantoins and N-carboxyphenylhydrazidoamino acids were optically inactive. This observation is in agreement with earlier reports that optically active hydantoins are rapidly racemized in basic solutions.[8]

For further confirmation of their structures, the N-carboxyphenylhydrazidoamino acids were oxidized to N-phenylazocarbonylamino acids (III) by the method

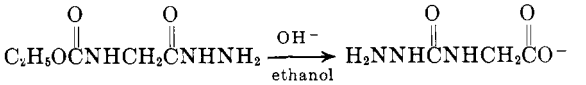


III

Pieroni[9] used to prepare phenylazoformamide (Table IV). The ultraviolet spectra of these compounds showed peaks at $\lambda_{max}^{CH_2Cl_2}$ 450.0 mμ (ε 112) and 297.5 mμ (ε 12,500). Grammaticakis[10] reported that the ultraviolet spectrum of phenylazoformamide showed peaks at λ_{max} 500.0 mμ (ε 170) and 300.0 mμ (ε 10,000). The infrared spectra were similar to those of the parent N-carboxyphenylhydrazidoamino acids. In each case, the N–H absorption at 3.08 μ was reduced, there was a shift of the urea carbonyl absorption from 6.01 to 5.90 μ, the absorption band at 6.32 μ (which has been attributed[3] to an anilino group) disappeared, and a new absorption band appeared at 8.38 μ.

The N-phenylazocarbonylamino acids (III) were stable under conditions that promote free-radical reactions. The ultraviolet spectra of their ethanol solutions remained unchanged after 24 hr. of refluxing in the presence of oxygen and after being irradiated for 24 hr. with ultraviolet light.

That the reaction was not restricted to phenylhydrazides was shown by the conversion of carboethoxy-



(6) D. G. H. Ballard and C. H. Bamford, *J. Chem. Soc.*, 355 (1958).
(7) M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).
(8) H. D. Dakin, *Am. Chem. J.*, **44**, 48 (1910); M. Bavarnick and H. T. Clarke, *J. Am. Chem. Soc.*, **60**, 2426 (1938).
(9) A. Pieroni, *Gazz. chim. ital.*, **53**, 32 (1922).
(10) P. Grammaticakis, *Bull. soc. chim. France*, [5] **20**, 86 (1953).

TABLE IV

N-PHENYLAZOCARBONYLAMINO ACIDS

N-Phenylazocarbonylamino acid	Yield, %	Recrystallized from	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Glycine	48	Chloroform	143–145	$C_9H_9N_3O_3$	52.17	51.95	4.34	4.23	20.20	20.12
DL-Alanine	70	Chloroform	139–141	$C_{10}H_{11}N_3O_3$	54.29	54.17	5.01	4.83	19.00	19.20
DL-Leucine	63.5	Ether	116–118	$C_{13}H_{17}N_3O_3$	59.30	59.11	6.51	6.68	15.96	15.83
DL-Phenylalanine	66.8	Chloroform	131–133	$C_{16}H_{15}N_3O_3$	64.64	64.58	5.09	5.02	14.13	14.18
DL-Tyrosine	58	Ether	158–159.5	$C_{16}H_{15}N_3O_4$	61.34	61.41	4.83	4.87	13.41	13.26

TABLE V

PROPERTIES OF N-CARBOXYPHENYLHYDRAZIDOAMINO ACID PHENYLHYDRAZIDES

N-Carboxyphenylhydrazidoamino acid	Acid, *M*	Phenylhydrazine, *M*	Papain, g./l.	Reaction time, days	Yield,[a] %	$[\alpha]^{25}D$	M.p., °C.
Glycine	0.02	0.14	16	8	65	...	201.5–203
DL-Alanine	0.03	0.52	5	8	43	+57.9[b]	206–207
DL-Leucine	0.02	0.19	16	7	55	+36.0[c]	205–206
DL-Phenylalanine	0.02	0.14	8	8	31	+25.5[d]	195.5–197
DL-Tyrosine	0.02	0.40	4	5	30	+18.8[e]	202–203

[a] Based on a racemic mixture of N-carboxyphenylhydrazido amino acid, after one recrystallization. [b] $[\alpha]^{25}D$ (*c* 1.00, glacial acetic acid). [c] $[\alpha]^{25}D$ (*c* 1.00, methanol). [d] $[\alpha]^{25}D$ (*c* 1.20, glacial acetic acid). [e] $[\alpha]^{25}D$ (*c* 2.00, glacial acetic acid).

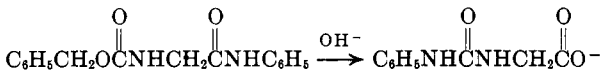
TABLE VI

ANALYSES OF N-CARBOXYPHENYLHYDRAZIDOAMINO ACID PHENYLHYDRAZIDES

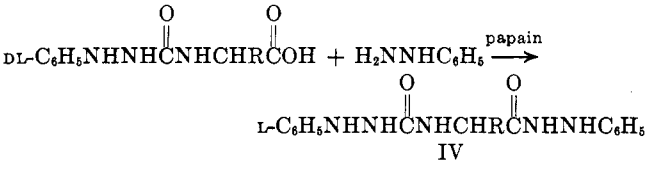
N-Carboxyphenylhydrazidoamino acid phenylhydrazide	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Glycine[a]	$C_{15}H_{17}N_5O_2$	60.18	59.92	5.73	5.76	23.40	23.67
DL-Alanine	$C_{16}H_{19}N_5O_2$	61.32	61.26	6.11	6.00	22.35	22.28
DL-Leucine	$C_{19}H_{25}N_5O_2$	64.20	64.07	7.09	6.92	19.71	19.90
DL-Phenylalanine	$C_{22}H_{23}N_5O_2$	67.85	67.70	5.95	6.03	17.99	18.01
DL-Tyrosine	$C_{22}H_{23}N_5O_3$	65.17	65.21	5.72	5.67	17.28	17.36

[a] Recrystallized from methanol–ether–ligroin (1:5:5).

glycine hydrazide to N-carboxyhydrazidoglycine and by the conversion of N-carbobenzoxyglycine anilide to phenylhydantoic acid.



As it has been shown that the nature of the acyl group has a profound effect upon the stereochemical course of the papain-catalyzed reaction of an N-acylated amino acid with phenylhydrazine forming the phenylhydrazide,[11] it was of interest to determine if the N-carboxy phenylhydrazidoamino acids could be used as substrates in the papain-catalyzed reaction with phenylhydrazine to form N-carboxyphenylhydrazido-L-amino acid phenylhydrazides and if the D-isomer would also



IV

react in this reaction. The N-carboxyphenylhydrazido derivatives of glycine, DL-alanine, DL-phenylalanine, DL-leucine, and DL-tyrosine reacted with phenylhydrazine in the presence of papain to form N-carboxyphenylhydrazido-L-amino acid phenylhydrazides (IV). The reactions proceeded readily with yields of up to 65% based on the L-antipode (Tables V and VI). From

(11) (a) E. L. Bennett and C. Niemann, *J. Am. Chem. Soc.*, **70**, 2610 (1948); (b) E. L. Bennett and C. Niemann, *ibid.*, **72**, 1798 (1950); (c) H. B. Milne and C.-H. Ping, *ibid.*, **79**, 645 (1957); (d) H. B. Milne and C. M. Stevens, *ibid.*, **72**, 1742 (1950); (e) H. B. Milne, S. L. Razniak, R. P. Bayer, and D. W. Fish, *ibid.*, **82**, 4582 (1960).

TABLE VII

ENZYMATIC SYNTHESIS OF N-CARBOXYPHENYLHYDRAZIDOLEUCINE PHENYLHYDRAZIDE

Fraction	Incubation, days	Yield[a] g.	Yield[a] %	M.p., °C.	$[\alpha]^{25}D$
1	0.4	0.48	11.9	172–179	+46.5
2	1.1	1.27	31.6	172–179	+45.9
3	2.0	1.66	41.3	173–180	+43.8
4	3.2	2.05	51.0	193–201	+38.3
5	7.2	2.14	53.2	198–208	−2.8

[a] Total yield, obtained by adding the fractions.

Table VII it is apparent that a small amount of N-carboxyphenylhydrazido-D-leucine phenylhydrazide was formed along with the L-antipode. This behavior is the same as has been observed previously when carboalkoxyamino acids and benzylthiocarbonylamino acids were used as substrates in the papain-catalyzed reaction with phenylhydrazine.[11]

It should be noted that the N-carboxyphenylhydrazido-L-amino acid phenylhydrazides (IV) have positive rotations. The carbobenzoxy-, carboallyloxy-, benzylsulfonyl-, and the benzylthiocarbonyl-L-amino acid phenylhydrazides which have been reported all have negative rotations.[11] The positive rotations may be due to conformations of the N-carboxyphenylhydrazido-L-amino acid phenylhydrazides resulting from internal hydrogen bonding.

When N-carboxyphenylhydrazido-L-leucine phenylhydrazide was oxidized with ferric chloride in aqueous solution, N-phenylazocarbonyl-L-leucine was isolated. In view of the stabilities of the phenylazocarbonylamino acids in aqueous solutions, the result of the ferric chlo-

TABLE VIII

N-CARBOALKOXYAMINO ACID PHENYLHYDRAZIDES

N-Carboalkoxy-α-amino acid	Reaction time, days	Yield,[a] %	M.p., °C.	Lit. m.p., °C.	$[\alpha]^{22}D$ (c 2, $CHCl_3$)	Lit. $[\alpha]^{25}D$
N-Carbobenzoxyglycine	6	87.5	142	144		
N-Carbobenzoxy-DL-alanine	3	67	152–154	154.5–155.5	−47.9	−27.2[b]
N-Carbobenzoxy-DL-leucine	8	66.8	139–140		−40.8	
N-Carbobenzoxy-L-leucine	2	68	139.5–140.5		−54.1	
N-Carboethoxy-DL-leucine	2	85.5	133–135		−67.2	
N-Carboethoxy-DL-phenylalanine	4	74	149–150	156.5–159.5	−24.3[c]	−22.2[d]
N-Carboallyloxy-DL-tyrosine	7	60	163–165	162–163	−10.0[e]	−8.5[f]
N-Carboethoxy-L-tyrosine	4	45	178–179		−17.4	

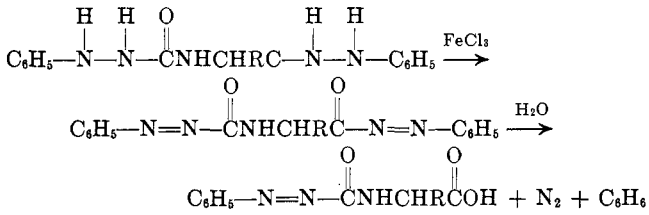
[a] Based on L-isomer only; after one recrystallization from ethanol–water. [b] c 5, acetone.[11b] [c] c 1, ethanol. [d] c 8, pyridine.[11b] [e] c 0.8, ethanol. [f] c 1, ethanol.[12]

TABLE IX

ANALYSES OF N-CARBOALKOXY-α-AMINO ACID PHENYLHYDRAZIDES

N-Carboalkoxy-α-amino acid phenylhydrazide	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
N-Carbobenzoxy-L-leucine	$C_{20}H_{25}N_3O_3$	67.58	67.78	7.09	6.85	11.82	11.56
N-Carboethoxy-L-leucine	$C_{15}H_{23}N_3O_3$	61.41	61.33	7.90	7.80	14.33	14.19
N-Carboethoxy-L-tyrosine	$C_{21}H_{25}N_3O_6$	60.71	60.50	6.07	5.95	10.11	10.14

ride oxidation was not unexpected; we had shown[12] previously that acylamino acid phenylhydrazides are oxidized by ferric chloride to the acylamino acids, nitrogen, and benzene. These results are also in agreement with the suggestion of Kelly[13] that phenylhydrazides are oxidized first to the azo compounds which then react with water with a heterolytic elimination of nitrogen.



This reaction was used in the preparation of a sample of nearly optically pure N-carboxyphenylhydrazido-L-leucine phenylhydrazide. N-Carboxyphenylhydrazido-L-leucine phenylhydrazide ($[\alpha]^{25}D$ +39.2), estimated to be 91% L-isomer, was oxidized in an aqueous solution of ferric chloride to give N-phenylazocarbonyl-L-leucine ($[\alpha]^{25}D$ −33.9). This was used as substrate in a papain-catalyzed reaction with phenylhydrazine. Excess cysteine was added to reduce the N-phenylazocarbonyl-L-leucine to N-carboxyphenylhydrazido-L-leucine. The product obtained, after 43.5% of the substrate had reacted, was N-carboxyphenylhydrazido-L-leucine phenylhydrazide ($[\alpha]^{25}D$ +46.97).

Experimental[14]

Enzymatic Syntheses of N-Carboalkoxy-α-amino Acid Phenylhydrazides.—The N-carboalkoxy-α-amino acid phenylhydrazides were prepared by the enzymatic method reported previously.[11] Their properties are listed in Table VIII and their analyses in Table IX.

N-Carboxyphenylhydrazidoamino Acids.—The N-carboxyphenylhydrazidoamino acids were prepared by the method of Milne and Fish.[2] Their properties and analyses are shown in Table I.

The N-carboxyphenylhydrazidoamino acids, although prepared from optically active N-carboalkoxyamino acid phenylhydrazides which were predominantly L-isomers, showed no optical activity.

The infrared spectra of N-carboxyphenylhydrazidoamino acids (solid film from dioxane) showed the following maxima: N–H stretch, 3.08; C=O stretch, 5.80 and 5.98; N–H deformation, 6.45; and C–H out-of-plane deformation, 13.20 and 14.40 μ. The ultraviolet spectra (95% ethanol solutions) showed peaks at λ_{max} 233 mμ (ε 12,400) and 282 mμ (ε 1600).

5-Substituted 3-Anilinohydantoins.—The N-carboalkoxy-α-amino acid phenylhydrazide (0.02 mole) was dissolved in 180 ml. of absolute ethanol which was previously saturated with nitrogen. Alcoholic potassium hydroxide (0.002 mole in 20 ml. of absolute ethanol) was added and the resulting solution was refluxed for 1–2 hr. under a nitrogen atmosphere. The solvent was removed with a rotary evaporator and the resulting oil was triturated with ether to give a slightly yellow crude product which was recrystallized from ethanol–water and washed with ether to yield a white solid.

The anilinohydantoins, prepared from optically active N-carboalkoxy-α-amino acid phenylhydrazides, which were predominantly L-isomers, showed no optical activity.

Infrared spectra of 5-substituted 3-anilinohydantoins (film deposited from chloroform) showed the following maxima: N–H stretch, 3.1; C=O stretch, 5.62 and 5.78; and N–H deformation, 6.23 μ. The ultraviolet spectra (absolute ethanol solutions) showed peaks at λ_{max} 228 mμ (ε 12,000) and 278 mμ (ε 1450). The properties and analyses of these products are shown in Table II.

Kinetics of the Hydrolysis of Some 5-Substituted 3-Anilinohydantoins.—A solution of 0.02 mole of the 5-substituted 3-anilinohydantoin in 180 ml. of absolute ethanol (saturated with nitrogen) was heated to reflux, and 0.02 mole of potassium hydroxide in 20 ml. of absolute ethanol was added; this gave initial concentrations of 0.1 *M* for both potassium hydroxide and hydantoin. The solution was refluxed (72–73°) under a nitrogen atmosphere. At appropriate intervals, 10-ml. aliquots were removed by pipet and titrated (phenolphthalein) with 0.1 *N* hydrochloric acid. The data for the reactions are shown in Table III.

Preparation of N-Phenylazocarbonylamino Acids.—The N-carboxyphenylhydrazido amino acids were oxidized to N-phenyl-

(12) H. B. Milne, J. E. Halver, D. So Ho, and M. S. Mason, *J. Am. Chem. Soc.* **79**, 637 (1957).

(13) R. B. Kelly, *J. Org. Chem.*, **28**, 453 (1963).

(14) All melting points are uncorrected. The microanalytical work was performed by the Galbraith Laboratories, Knoxville, Tenn. The infrared spectra were determined with a Beckman IR-5 spectrophotometer and were obtained from samples deposited on rock salt plates from the solvents listed; a general procedure for this method is discussed by Randall.[15] The ultraviolet spectra were determined with a Cary Model 14 recording spectrophotometer using 1-cm. quartz cells, with 95% ethanol or absolute ethanol as the solvent. The papaya latex used in these experiments was kindly furnished by the Wallerstein Co., Staten Island, N. Y.

(15) H. M. Randall, R. G. Fowler, N. Fuson, and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949.

azocarbonyl amino acids by the method of Pieroni.[5] Their properties and analyses are shown in Table IV.

The N-carboxyphenylhydrazidoamino acid (0.01 mole) and 0.01 mole of sodium bicarbonate were dissolved in 80 ml. of water. This solution was added to a solution of potassium permanganate (0.005 mole) in 20 ml. of water, and the resulting solution was stirred for 9 min. Oxalic acid (0.01 mole, dissolved in 50 ml. of water) was added to the above solution, and this was then made acidic by the dropwise addition of dilute sulfuric acid. The acidic solution was extracted with five 25-ml. portions of ether. The ether extract was dried over anhydrous magnesium sulfate, and the solvent was evaporated to yield a red-orange solid which was recrystallized from the solvent indicated in Table IV.

The infrared spectra of the N-phenylazocarbonylamino acids (films deposited from methanol) showed the following maxima: N–H stretch at 3.08 μ was reduced as compared with the starting material; C=O stretch [NHC(O)NH] was shifted from 6.01 to 5.90 μ; N–H deformation at 6.23 μ disappeared, and a new peak appeared at 8.38 μ. The ultraviolet spectra (dichloromethane solutions) showed peaks at λ_{max} 450.0 mμ (ϵ 112) and 297.5 mμ (ϵ 12,500).

Enzymatic Syntheses of N-Carboxyphenylhydrazidoamino Acid Phenylhydrazides.—The N-carboxyphenylhydrazidoamino acid phenylhydrazides were prepared enzymatically by the method reported previously.[11] Their physical properties are listed in Table V and their analyses are given in Table VI.

The infrared spectra of N-carboxyphenylhydrazidoamino acid phenylhydrazides (films deposited from dioxane) showed maxima at N–H stretch, 3.08; C=O stretch, 6.00; N–H deformation, 6.30; and C–H out-of-plane deformation, 13.25 and 14.40 μ. The ultraviolet spectra of these compounds (absolute ethanol solutions) showed peaks at λ_{max} 235 mμ (ϵ 21,200) and 282 mμ (ϵ 3000). Although all N-carboxyphenylhydrazidoamino acids used were optically inactive, the products (excepting the glycine derivative) were strongly dextrorotatory.

Enzymatic Synthesis of N-Carboxyphenylhydrazido-L-leucine Phenylhydrazide.—N-Carboxyphenylhydrazido-DL-leucine (3.0 g., 0.011 mole) was dissolved in 100 ml. of water with an equivalent amount of sodium hydroxide, and this solution was added to a solution of 14.4 g. (9.1 mole) of phenylhydrazine hydrochloride, 4 g. of L-cysteine hydrochloride, and 3 g. of sodium versenate in 100 ml. of water. The volume of the solution was brought to 500 ml. with 2 *M* sodium acetate–acetic acid buffer (pH 4.7), 5 g. of papain was added, and the solution was saturated with nitrogen and incubated at 40°. At intervals the solid product was collected by filtration, and the filtrate was incubated further. The melting point and specific rotation of each sample of precipitate were determined. The results are shown in Table VII.

N-Phenylazocarbonyl-L-leucine.—N-Carboxyphenylhydrazido-L-leucine phenylhydrazide (1 g., 0.0028 mole), $[\alpha]^{25}D$ +39.2° (*c* 1.3, glacial acetic acid), was dissolved in 100 ml. of acetone, the solution was heated to 40°, and a solution of ferric chloride hexahydrate (8 g., 0.03 mole, in 30 ml. of water) was added dropwise over a period of 40 min. The acetone was removed with a rotary evaporator, and the remaining oily aqueous layer was extracted with three 50-ml. portions of ether. The ether solution was dried over anhydrous magnesium sulfate, and the ether was evaporated to give an oil. The oil did not crystallize, but it had an infrared spectrum identical with that of a sample of N-phenylazocarbonyl leucine prepared by oxidizing N-carboxyphenylhydrazido-DL-leucine. The yield was 0.4 g. (54%), $[\alpha]^{25}D$ −33.9° (*c* 1.78, methanol).

N-Phenylazocarbonyl-L-leucine as Substrate in Enzymatic Synthesis.—N-Phenylazocarbonyl-L-leucine (0.34 g., 0.0013 mole), $[\alpha]^{25}D$ −33.9° (*c* 1.78, methanol), was dissolved in 25 ml. of dilute sodium hydroxide solution, and this was added to a solution of phenylhydrazine hydrochloride (5 g., 0.035 mole) and 2 g. of L-cysteine hydrochloride in 50 ml. of water, and the volume of the solution was brought to 275 ml. The solution was buffered at pH 4.7 with approximately 1 *M* sodium acetate and acetic acid; 1 g. of versene and 1 g. of papain were added, and the solution was saturated with nitrogen and incubated at 40°. The product was isolated after 4 days and recrystallized from acetone–ether. The yield was 0.2 g. (43.5%), m.p. 186–188.5°, $[\alpha]^{25}D$ +46.97° (*c* 1.88, glacial acetic acid). The infrared spectrum was identical with that of a sample of N-carboxyphenylhydrazido-L-leucine phenylhydrazide prepared from N-carboxyphenylhydrazido-DL-leucine.

Phenylhydantoic Acid.—N-Carbobenzoxyglycine anilide (2.84 g., 0.01 mole) was refluxed in 50 ml. of 0.2 *N* alcoholic potassium hydroxide, under a nitrogen atmosphere, for 4 hr. The solvent was removed with a rotary evaporator, the remaining oil was dissolved in 25 ml. of water, and the product was precipitated by acidification and recrystallized from ethanol; the yield was 1.8 g. (95%), m.p. 186–188°. A mixture (m.p. 187–189°) showed no depression, and the infrared spectrum was identical with that for an authentic sample of phenylhydantoic acid.

Potassium N-Carboxyhydrazidoglycinate.—N-Carboethoxyglycine (15 g., 0.1 mole) was dissolved in 150 ml. of absolute methanol, and the solution was saturated with dry hydrogen chloride gas and stored at room temperature for 4 days. The solvents were removed, and the resulting oil was dissolved in 25 ml. of absolute ethanol; 5 ml. (0.08 mole) of 85% hydrazine hydrate was added, and the resulting solution was stored for 3 days at room temperature. The solvents were removed, and the resulting oil was treated three times, by the addition and subsequent evaporation under vacuum, of three portions of 15–20 ml. of absolute ethanol. The resulting oil was dissolved in 100 ml. of ethanol, and 5.6 g. (0.1 mole) of potassium hydroxide was added. This solution was refluxed, for 4 hr., and then cooled, and the salt was separated by filtration and washed with hot methanol; the yield was 11.3 g. (65%), m.p. 212–214°.

Anal. Calcd. for $C_3H_6KN_3O_3$: C, 21.05; H, 3.55; N, 24.54. Found: C, 21.20; H, 3.57; N, 24.84.

The Reaction of 1,1-Bis(diethylamino)ethene with Phenylmethanesulfonyl Chloride

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Phenylmethanesulfonyl chloride reacts with 1,1-bis(diethylamino)ethene to give a substitution product, whereas ketene diethyl acetal yields a cycloadduct. Some of the factors which appear to be involved in determining the nature of products are discussed.

In the presence of base, various sulfonyl chlorides react with ketene diethyl acetal to give thietane dioxides (referred to hereafter as cycloadducts).[1] However, under similar conditions, ketene aminals yield either a cycloadduct or a substitution product depending on the nature of the reactants and the reaction media.[2,3] This paper attempts to describe some factors which bring about the substitution products.

In tetrahydrofuran, 1,1-bis(diethylamino)ethene reacted with phenylmethanesulfonyl chloride in the presence of triethylamine, to produce a substitution

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