105.5°) on recrystallization from carbon tetrachloride. The best conditions for employing this catalytic brominolysis are being developed in connection with another study in progress in our Laboratory.

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Summary

The chlorinolysis of 2,4-dinitrophenyl disulfide is catalyzed by various metal halide catalysts, as well as by sulfuric acid. The application of the catalytic action to the synthesis of 2,4-dinitrobenzenesulfenyl chloride is described. Oxygen-containing substances, such as water, ether or phenol, which preferentially coördinate the catalysts, inhibit the catalytic effects.

The brominolysis of 2,4-dinitrophenyl disulfide was also found to be catalyzed by aluminum bromide.

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[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 1314]

The Enzymatic Synthesis of N-Acyl-D- and L-Phenylalanylphenylhydrazides[1]

BY EDWARD L. BENNETT[2] AND CARL NIEMANN

The papain catalyzed synthesis of acylated α-amino acid hydrazides,[3] conducted at approximately *p*H 5, may be described in part by the equation

$$RCONHCHR'CO_2^- + R''NHNH_2 \longrightarrow RCONHCHR'CONHNHR'' + OH^-$$

since amide or hydrazide formation has never been observed with amines which are substantially protonated at *p*H 5[4] whereas all of the N-acylated-α-amino acids are almost completely ionized at this *p*H. With regard to the stereochemical aspect of the above reaction it has been assumed that only the N-acyl-L-amino acid amide or hydrazide is formed[3] though it has been suggested[5] that when R′ is small, *i. e.*, a methyl group, there may be some loss in enzymatic stereochemical specificity.

The first indication that factors other than the configuration about the asymmetric α-carbon atom and possibly the size of the R′ group in the N-acylated α-amino acid could influence the stereochemical course of the above reaction was obtained when it was observed[6] that N-carbobenzoxy-*o*-fluoro-DL-phenylalanine gave, in addition to the expected N-carbobenzoxy-o-fluoro-L-phenylalanylphenylhydrazide, significant quantities of N-carbobenzoxy-*o*-fluoro-D-phenylalanylphenylhydrazide. In continuing our studies along these lines we now wish to report some observations which clearly show that the nature of the acyl group, present in acylated DL-phenylalanines, may have a profound effect upon the stereochemical course of the papain catalyzed synthesis of the corresponding phenylhydrazides.

Five acylated DL-phenylalanines were incubated with activated papain, cysteine, and phenylhydrazine, at *p*H 4.6 and 40°, with an initial mole ratio of phenylhydrazine to DL-acid of 0.5. The precipitated phenylhydrazides were collected, additional phenylhydrazine added to the solutions, and the process repeated until no more phenylhydrazide was obtained. The specific rotations of all of the phenylhydrazide fractions were determined and the amount of L- or D-isomer present in each fraction computed from the specific rotation of the component L- and D-phenylhydrazides obtained by fractional crystallization of the crude hydrazides. The data obtained from these experiments are given in Table I. In a second series of experiments the effect of increasing the enzyme concentration from 0.3 to 2.5% or of increasing the initial mole ratio of phenylhydrazine to DL-acid from 0.5 to 1.0 on the reaction of acetyl-DL-phenylalanine with phenylhydrazine was studied. In either case no evidence was obtained for the formation of acetyl-D-phenylalanylphenylhydrazide (*cf.* Table I).

The above data clearly show that for an acylated DL-phenylalanine, $RCONHCH(CH_2C_6H_5)CO_2H$, when R = CH_3– or C_6H_5– the papain catalyzed synthesis of the phenylhydrazide proceeds with almost complete stereochemical specificity for the L-antipode. However, when R = CH_3O–, C_2H_5O– or $C_6H_5CH_2O$– this stereochemical specificity is lost to a striking degree and although the L-antipode is converted into the phenylhydrazide at the more rapid rate the rate of formation of the phenylhydrazide of the D-antipode is apparently of comparable magnitude. At present there are insufficient data to attempt to give a complete explanation of the above phenomena though it is now clear that a third factor must be added to the other two that previously had been believed to be sufficient to account for the stereochemical specificity of the proteolytic enzymes.

(1) Presented in part at the 115th meeting of the American Chemical Society at San Francisco, March, 1949.

(2) Procter and Gamble Fellow in Chemistry 1948-1949; present address, Radiation Laboratory, University of California, Berkeley, California.

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

(4) Unpublished experiments of Dr. P. L. Nichols.

(5) M. Bergmann, L. Zervas and J. S. Fruton, *J. Biol. Chem.*, **115**, 593 (1936).

(6) E. L. Bennett and C. Niemann, THIS JOURNAL, **70**, 2610 (1948).

TABLE I

ENZYMATIC SYNTHESIS OF D AND L-ACYLPHENYLALANYLPHENYLHYDRAZIDES, $RCONHCH(CH_2C_6H_5)CONHNHC_6H_5$

R =	% L-isomer in phenylhydrazide[a] F-1	F-2	F-3	F-4	Yield of phenylhydrazide, %[b] F-1 D	F-1 L	F-2 D	F-2 L	F-3 D	F-3 L	F-4 D	F-4 L	Total D	Total L
CH_2O–[c]	86	86	55		9	51	4	21	7	8			20	80
C_2H_5O–[d]	84	57	9	1[e]	14	74	15	21	26	3	17	0	72	98
C_2H_5O–[f]	80	57	9	1[e]	19	75	16	22	31	3	17	0	83	100
$C_6H_5CH_2O$–[g]	79	38	7		21	78	39	24	23	2			83	104
C_6H_5–[h]	96	95			3	76	1	21					4	97
CH_3–[i]	98	99	97		1	60	0	20	0	2			3	82
CH_3–[j]	100				0	63	0	0					0	63
CH_3–[k]	99	99												
CH_3–[l]	100				0	85	0	0					0	85

[a] Estimated from specific rotations of fractions. [b] Estimated from weights and specific rotations of fractions. [c] Initially 0.048 mole of DL-acid (I), 0.024 mole of phenylhydrazine (II), 0.025 mole of cysteine hydrochloride (III) and 4.4 g. of papain (IV) in 1000 ml. of solution 0.2 *F* in buffer; F-1 collected after 96 hr., 0.033 mole II and 0.019 mole III added, F-2 collected after additional 48 hr., F-3 after additional 7 days. [d] Initially 0.052 mole of I, 0.026 mole of II, 0.025 mole of III, and 2.0 g. of IV in 1000 ml. of solution 0.1 *F* in buffer; F-1 collected after 104 hr., 0.025 mole of II added, F-2 collected after additional 72 hr., F-3 after additional 16 days. [e] Crude D-acid from filtrate of F-3 (first expt.) combined with crude D-acid from second expt. and incubated for 56 hr. with 0.037 mole of II, 0.076 mole of III, and 1.0 g. of IV to give F-4. Yield of F-4 in each experiment assumed to be proportional to initial concentrations of I. [f] Initially 0.043 mole of I, 0.023 mole of II, 0.025 mole of III, and 2.0 g. of IV in 1000 ml. of solution 0.1 *F* in buffer; F-1 collected after 96 hr., 0.023 mole of II added, F-2 collected after additional 72 hr., F-3 after additional 16 days. [g] Initially 0.047 mole of I, 0.024 mole of II, 0.089 mole of III, and 9.7 g. of IV in 3800 ml. of solution 0.1 *F* in buffer; F-1 collected after 88 hr., 0.026 mole of II added, F-2 collected after additional 4 days, 0.009 mole II added, F-3 collected after additional 5 days. [h] Initially 0.039 mole of I, 0.020 mole of II, 0.089 mole of III, and 9.7 g. IV in 3800 ml. of solution 0.1 *F* in buffer; F-1 collected after 88 hr., 0.020 mole of II added, F-2 collected after additional 90 hr. [i] Initially 0.048 mole of I, 0.025 mole of II, 0.005 mole of III, and 0.62 g. of IV in 200 ml. of solution 0.5 *F* in buffer; F-1 collected after 85 hr., 0.025 mole of II added, F-2 collected after an additional 96 hr., F-3 after an additional 16 days. [j] Initially same as for *i* except 0.041 mole of III and 5.0 g. of IV; F-1 collected after 87 hr., no further precipitation. [k] Initially 0.024 mole of I, 0.027 mole of II, 0.003 mole of III and 0.31 g. of IV in 100 ml. of solution 0.5 *F* in buffer; F-1 collected after 88 hr., 0.013 mole of II added, F-2 collected after an additional 4 days. [l] Initially 0.13 mole of I, 0.13 mole of II, 0.012 mole of III and 2.2 g. of IV in 500 ml. of solution 0.5 *F* in buffer; F-1 collected after 88 hr., 0.025 mole of II and 0.006 mole of III added, no further precipitation in 50 hr.

TABLE II

PREPARATION OF N-ACYLATED-DL-PHENYLALANINES

Acyl group	Method of preparation	Yield, %	M. p., °C. Found	M. p., °C. Lit.
CH_3CO–	Ac_2O, NaOH; recryst. H_2O	88	152–154	151–152[9]
C_6H_5CO–	PhCOCl, NaOH; recryst. H_2O	91	185–188	187–188[10]
$C_6H_5CH_2OCO$–	BzOCOCl, NaOH; recryst. $PhCH_3$	80	103–104	103[11]
C_2H_5OCO–	EtOCOCl, NaOH; recryst. H_2O	56[a]	73–75	.....
CH_3OCO–	MeOCOCl, NaOH	97[b]	.....	.....

[a] *Anal.* Calcd. for $C_{12}H_{15}O_4N$ (237): C, 60.8; H, 6.4; N, 5.9. Found: C, 60.9; H, 6.2; N, 6.1. [b] Sirup, could not be crystallized.[12]

In view of the fact that carbobenzoxy-DL-alanine in the presence of activated papain and phenylhydrazine formed only the carbobenzoxy-L-alanylphenylhydrazide,[6] studies are now in progress to determine what combinations of amino acid side chains and acyl groups will result in a retention or loss of stereochemical specificity not only in the enzyme catalyzed formation of phenylhydrazides and anilides of acylated α-amino acids but also in the enzyme catalyzed hydrolysis of the corresponding amides and esters.[7]

Experimental[8]

N-Acylated DL-Phenylalanines.—The method of preparation and properties of the five N-acyl-DL-phenylalanines used in this study are summarized in Table II.

Papain Preparation.—Fifty grams of "Hygrade Papain" (Wallerstein Laboratories) rapidly ground in a cold mortar to a fine powder was suspended in 200 ml. of cold water, the suspension stirred at 4° for four hours, filtered under suction through a Whatman No. 1 paper, the clear yellow filtrate placed in an ice-bath, a rapid stream of hydrogen sulfide passed through the solution for three hours, the suspension centrifuged at 2000 r.p.m. for twenty minutes, and sufficient cold methanol added, slowly and with stirring, to the supernatant solution to bring the methanol concentration to 70 volume %. The precipitate was collected by centrifugation for twenty minutes at 2000 r.p.m., dissolved in 200 ml. of cold water, the solution saturated with hydrogen sulfide, centrifuged and the enzyme again precipitated with methanol. After four reprecipitations a product was obtained in 25–30% yield which was freely soluble in water and in an acetic acid–acetate buffer of *p*H 4.6.

Enzyme Experiments.[13]—The details of the various enzyme experiments are summarized in Table I. It should be added that a sodium acetate–acetic acid buffer

(7) S. Kaufman, H. Neurath and G. Schwert, *J. Biol. Chem.*, **177**, 793 (1949).

(8) All melting points are corrected.

(9) M. Bergmann and P. Stern, *Ber.*, **63**, 437 (1930).

(10) E. Fischer and A. Mouneyrat, *ibid.*, **33**, 2383 (1900).

(11) M. Bergmann and L. Zervas, *ibid.*, **65**, 1192 (1932).

(12) H. Leuchs and W. Geiger, *ibid.*, **41**, 1721 (1908).

(13) Complete protocols for each experiment may be found in the Ph.D. Thesis of E. L. Bennett, Calif. Inst. of Tech., Pasadena, 1949.

TABLE III

PROPERTIES OF PHENYLALANINE DERIVATIVES OBTAINED FROM ENZYME EXPERIMENTS

Compound[a]	M. p., °C.	$[\alpha]^{25}D$[b]	Analyses, % Calculated C	H	N	Found C	H	N
Phenylhydrazides								
CH_3OCO-L-	170–174	−23.8	65.2	6.1	13.4	65.2	6.1	13.3
CH_3OCO-DL-	181–182.5	− 0.2	65.2	6.1	13.4	64.9	6.0	13.7
C_2H_5OCO-L-	156.5–159.5	−22.2			12.8			12.6
C_2H_5OCO-D-	156–160.5	+23.4			12.8			12.8
C_2H_5OCO-DL-	171–172.5	− 0.4	66.0	6.5	12.8	66.2	6.7	12.7
$C_6H_5CH_2OCO$-L-	177–179	−24.6[i]	70.9	6.0	10.8	71.1	6.2	10.4
$C_6H_5CH_2OCO$-D-	178–179	+24.4[j]	70.9	6.0	10.8	71.1	6.0	10.9
$C_6H_5CH_2OCO$-DL-	159.5–161	0.0[k]	70.9	6.0	10.8	70.7	6.0	10.7
C_6H_5CO-L-[b]	215–217	−61.9	73.5	5.9	11.7	73.7	6.0	11.8
CH_3CO-L-[b]	207–208[e]	−34.6[l]	68.7	6.4	14.1	68.4	6.5	13.9
Acids								
C_6H_5CO-D-[c]	139.5–140.5[f]	+23.8[m]	71.4	5.6	5.2	71.6	5.9	5.0
CH_3CO-D-[d]	171–172[g]	−32.9[k,n]	63.8	6.3	6.8	63.9	6.4	6.6

[a] Obtained from crude hydrazide by fractional crystallization from toluene unless otherwise noted. [b] By fractional crystallization from ethanol. [c] Recrystallized from dilute aqueous hydrochloric acid. [d] Recrystallized alternately from ethanol and from water. [e] Lit.,[3] m. p. 205°. [f] Lit.,[10] m. p. 145–146°. [g] Lit.,[14] m. p. 172°. [h] c = 8% in pyridine unless otherwise noted. [i] $[\alpha]^{25}D$ −29.2° (c = 2.5% in chloroform). [j] c = 7% in pyridine. [k] c = 9% in pyridine. [l] Lit.,[3] $[\alpha]^{23}D$ −33.5° (c = 4.5% in pyridine). [m] $[\alpha]^{25}D$ −18.0° (c = 8% in 0.4 F NaOH); lit.,[10] $[\alpha]^{20}D$ −17.1° (c = 7% in 1 F NaOH). [n] $[\alpha]^{25}D$ −46.0° (c = 8% in ethanol); lit.,[14] $[\alpha]^{25}D$ −51° (in ethanol); for L-isomer,[15] $[\alpha]^{25}D$ +47.6° (in ethanol).

was used and that the pH was readjusted to 4.6 after the collection of each fraction. The weight, m. p. and specific rotation of each fraction was determined and the fractions then fractionally crystallized from suitable solvents in order to determine the melting points and specific rotations of each of the components of the various fractions. These data are summarized in Table III. The L-isomers were obtained from L-DL mixtures (initial fractions) and the D-isomers from D-DL mixtures (final fractions). The D-acids (*cf.* Table III) were obtained by acidification of the reaction mixture, extraction with ether and subsequent crystallization from the indicated solvents. The amount of L-isomer present in each fraction (*cf.* Table I) was estimated from the specific rotation of each fraction and the specific rotation of one or both components (*cf.* Table III).

(14) V. du Vigneaud and C. Meyer, *J. Biol. Chem.*, **98**, 295 (1932).

(15) R. Jackson and W. Cahill, *ibid.*, **126**, 37 (1938).

Summary

It has been shown that stereochemical specificity in the papain-catalyzed synthesis of phenylhydrazides of acylated phenylalanines is in part determined by the nature of the acyl group present in the acylated phenylalanines.

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[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 1319]

The Preparation and Resolution of the Three Isomeric Nuclear Substituted Monofluoro-DL-phenylalanines

BY EDWARD L. BENNETT[1] AND CARL NIEMANN

The observation that *m*-fluoro-DL-phenylalanine may effectively inhibit the metabolism of phenylalanine by a competitive process[2] suggested the desirability of extending these studies to include all of the isomeric nuclear substituted monofluorophenylalanines. The three nuclear substituted monofluoro-DL-phenylalanines had been prepared previously by the condensation of the appropriate fluorobenzaldehydes with hippuric acid,[3,4] the former compounds being obtained by chromyl chloride oxidation of the corresponding fluorotoluenes, or by hydrolysis of the fluorobenzal chlorides. The over-all yields from toluidine to the amino acid were 2.3, 7.0 and 5.0% for the *o*-, *m*- and *p*-fluoro-DL-phenylalanines, respectively, or 3.1, 10.3, and 9.5% from the corresponding fluorotoluenes.[5] The above yields were not substantially improved when the fluorobenzaldehydes were prepared from the amino- or nitrobenzoic acids *via* the McFadyen–Stevens reaction.[6] However, when the isomeric monofluorotoluenes were converted into the corresponding monofluorobenzyl chlorides by a vapor

(1) Procter and Gamble Fellow in Chemistry 1948-1949; present address: Radiation Laboratory, University of California, Berkeley, California.

(2) H. K. Mitchell and C. Niemann, THIS JOURNAL, **69**, 1232 (1947).

(3) G. Schiemann and W. Roselius, *Ber.*, **65**, 1439 (1932).

(4) J. Mead, Ph.D. Thesis, Calif. Inst. Tech., 1942.

(5) G. Schiemann, *Z. physik. Chem.*, **A156**, 397 (1931).

(6) J. S. McFadyen and T. S. Stevens, *J. Chem. Soc.*, 584 (1936).