Anal. Calcd for $C_{24}H_{24}N_2O_{11}S_2$: C, 49.66; H, 4.17; N, 4.83. Found: C, 49.91; H, 4.33; N, 5.11.

Reaction of 1-(5′-*O*-Benzoyl-2′-*O*-mesyl-3′-*O*-tosyl-β-D-lyxofuranosyl)uracil (7) with Sodium Benzoate.—A mixture of **7** (360 mg, 0.62 mmol) and sodium benzoate (270 mg, 1.86 mmol) in DMF (4 ml) was stirred at 100° for 20 min. Thin layer chromatography with an aliquot of the reaction mixture indicated one main product and essentially no starting material. The mixture was poured into ice–water (50 ml) and extracted with ethyl acetate (2 × 50 ml). The ethyl acetate solution was dried with sodium sulfate and evaporated to a foam, which was submitted to preparative thin layer chromatography using silicic acid and chloroform–ethyl acetate (1:1) to give 37 mg (18%) of a crystalline substance, mp 192–194°. Its identity with **6** was confirmed by ir and uv spectroscopy.

1-(5′-*O*-Benzoyl-2′,3′-di-*O*-mesyl-β-D-lyxofuranosyl)uracil (9).—A solution of 1-(5′-*O*-benzoyl-β-D-lyxofuranosyl)uracil (**1**) (0.32 g, 0.92 mmol) in dry pyridine (3 ml) was treated with mesyl chloride (0.17 ml, 2.2 mmol) at 0° overnight and the mixture was worked up as in the case of compound **7**. The finally obtained pasty product contained trace amounts of impurities as indicated by tlc. Preparative thin layer chromatography with the use of silica gel and ethyl acetate as developer gave 0.36 g (78%) of a homogeneous foam, which was used as such for the next elimination reaction, nmr ($CDCl_3$) δ 3.15 (6 H, d, two mesyl).

Reaction of 1-(5′-*O*-Benzoyl-2′,3′-di-*O*-mesyl-β-D-lyxofuranosyl)uracil (9) with Sodium Benzoate.—A mixture of **9** (0.36 g, 0.72 mmol) and sodium benzoate (270 mg, 3.6 molar equiv) in DMF (5 ml) was stirred at 110°. Thin layer chromatography with the use of an aliquot of the reaction mixture indicated that over 50% of the starting material was converted to another faster moving substance after 15 min of reaction. After 35 min, the rest of the starting material was remarkably reduced with the appearance of a slight amount of a new product and with increase in resinous substances. It took totally 2 hr of stirring for the complete disappearance of the starting material. The mixture was now evaporated *in vacuo* as far as possible and the residue was extracted with ethyl acetate (4 × 30 ml) in the presence of water (15 ml). The ethyl acetate solution was dried with sodium sulfate and evaporated to a paste, which was submitted to preparative thin layer chromatography with the use of silica gel and ethyl acetate. Elution of the main band with ethyl acetate gave a crystalline substance, mp 192–193° (50 mg, 21%), whose identity with an authentic sample of **6** was confirmed by ir spectra and the mixture melting point determination.

1-(2′-*O*-Tosyl-β-D-lyxofuranosyl)uracil (10).—Compound **4** (0.3 g, 0.517 mmol) was combined with saturated ethanolic ammonia (16 ml) in a pressure tube, which was heated in an oil bath at 100–105° for 16 hr. After cooling, the solvent and excess ammonia were evaporated and the solid residue was crystallized from methanol to give 94 mg (45%) of colorless needles of **10**: mp 259–261°; λ_{max}^{EtOH} 225 nm (ε 14,000) and 260 (9100); nmr (DMSO-d_6) δ 2.43 (3 H, s, methyl in the tosyl group), 3.6 (3 H, br m, 2 $H_{5'}$ + $H_{4'}$), 4.15 (1 H, dd, $J_{2',3'}$ = 4.7, $J_{3',4'}$ = 3.7 Hz, $H_{3'}$), 5.25 (1 H, dd, $J_{1',2'}$ = 6.8, $J_{2',3'}$ = 4.7 Hz, $H_{2'}$), 5.6 (1 H, d, $J_{5,6}$ = 8 Hz, H_5), 6.15 (1 H, d, $J_{1',2'}$ = 6.8 Hz, $H_{1'}$), and 7.2–7.8 (5 H, m, aromatic protons containing H_6).

Registry No.—**1**, 38359-50-1; **2**, 38359-51-2; **3**, 38359-52-3; **4**, 38359-53-4; **5**, 38359-54-5; **6**, 38359-55-6; **7**, 38431-66-2; **9**, 38431-65-1; **10**, 38359-56-7.

The Use of Papain in Resolving Racemic *N*-(Alkoxycarbonyl)glycines and *N*-(Alkoxycarbonyl)alanines That Contain Small Alkoxy Groups[1]

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Papain promoted very rapid reactions at pH 4.5 between small molecular weight *N*-(alkoxycarbonyl)amino acids and *m*- or *p*-anisidine. Hindrance toward reactions was evident when ortho-substituted anilines were used. For *N*-(*tert*-butoxycarbonyl)- and *N*-(*tert*-pentyloxycarbonyl)-DL-alanines, resolution amounted to ~95 to 100%. A few *N*-(alkoxycarbonyl)glycines were used in which the asymmetric center was placed in the alkoxycarbonyl group. These groups included (*R,S*)-*N*-(*sec*-butoxycarbonyl), (*R,S*)-*N*-(1-methylbutoxycarbonyl), (*R,S*)-*N*-(2-methylbutoxycarbonyl), and (*S*)-*N*-(2-methylbutoxycarbonyl). A preference for one enantiomer was shown for each racemic mixture investigated. Anisidides formed from (*R,S*)-*N*-(2-methylbutoxycarbonyl)glycine displayed a preponderance of the *S* enantiomer to the extent of ~56% after an early period of incubation. This conclusively demonstrated the ability of papain to exert a modest stereochemical control, even though the asymmetric center is removed four or five atoms away from its usual position in *N*-acyl-DL-amino acids.

N-(*tert*-Butoxycarbonyl)- and *N*-(*tert*-pentyloxycarbonyl)amino acids have been used in solid-phase peptide syntheses of bradykinin,[2] ferredoxin,[3] ribonuclease,[4] and human growth hormone.[5] Although papain has been used to catalyze the synthesis of anilides of many *N*-acylamino acids,[6] anilides of low molecular weight *N*-(alkoxycarbonyl)amino acids have not been prepared in this manner. It was the purpose of the present research to explore the use of papain as a catalyst for reactions between a few substituted anilines and such *N*-acylamino acids, which contain only four or five carbons in the alkoxy group. By placing an asymmetric center in the alkoxy group of *N*-(alkoxycarbonyl)glycines, the zone of stereochemical control exerted by papain would be substantially altered and a considerably different perspective would therefore be achieved.

Four principal objectives were attained through this research. First, the use of *N*-*tert*-alkoxycarbonyl derivatives of glycine, DL-alanine, and L-alanine permitted a comparison to be made of their relative rates of reactions with known rates of more familiar *N*-acyl derivatives of these same amino acids. Second, by careful selection of ortho-, meta-, and para-substituted anilines, the effect of position and kind of substituent on the ability of this type of base to participate in such reactions was disclosed. Third, the extent of resolution of racemic *N*-(alkoxycarbonyl)amino acids was revealed through comparison of specific rotations of their reaction products with specific rotations of corresponding products from single enantiomers of the given *N*-acyl-

(1) Presented before the Pacific Conference on Chemistry and Spectroscopy, Tenth Pacific Meeting, Seventh Western Regional Meeting, Anaheim, Calif., Oct 19, 1971.

(2) R. B. Merrifield, *J. Amer. Chem. Soc.*, **86**, 304 (1964).

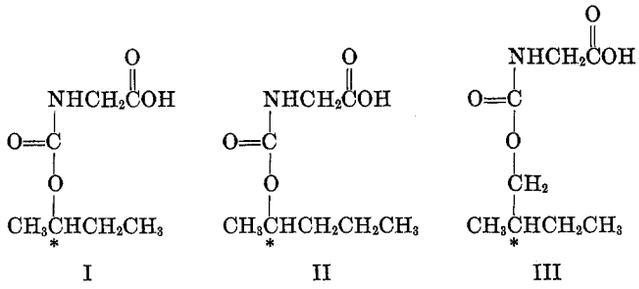
(3) E. Bayer, C. Jung, and H. Hagemeir, *Tetrahedron*, **24**, 4853 (1968).

(4) B. Gutte and R. B. Merrifield, *J. Biol. Chem.*, **246**, 1922 (1971).

(5) (a) C. H. Li and D. Chung, *Int. J. Protein Res.*, **3**, 73 (1971); (b) W. Danho and C. H. Li, *ibid.*, **3**, 81, 99 (1971); (c) K. Kovacs, Y. Kovacs-Petres, and C. H. Li, *ibid.*, **3**, 93 (1971).

(6) (a) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937); (b) D. G. Doherty and E. A. Popenoe, Jr., *ibid.*, **189**, 447 (1951).

amino acids. Fourth, the most significant feature of this investigation was to subject (*R,S*)-*N*-(*sec*-butoxycarbonyl)glycine (I), (*R,S*)-*N*-(1-methylbutoxycarbonyl)glycine (II), and (*R,S*)-*N*-(2-methylbutoxycarbonyl)glycine (III) to papain-catalyzed reactions with



I II III

m- and *p*-anisidines. The asymmetric center (*) was removed either four or five atoms away from the customary position next to the carboxyl group.

Results and Discussion

An interpretation of the results of these and other experiments has been made easier because many obscure points concerning papain have been cleared up. In other instances, the obscurity has increased and requires clarification by further research. Numerous experiments have progressively exposed the nature of papain's catalytic activity and the types of reactions that papain can foster. Its chirality and conformation provide features for unique stereochemical control. It is known that the mercapto group, –SH, of cysteine residue 25, counted from the amino terminal, can form thio esters, –SCOR,[7,8] on exposure to appropriate substrates. These include *N*-acylamino acids,[9] esters,[10] amides,[11] polypeptides,[12] or proteins.[13] A dilobal, spheroidal conformation[14] has emerged from an elucidation of the complete primary structure of the 212 amino acid residues of papain, combined with a rigorous X-ray analysis. This has afforded a more sophisticated basis for probing into many of the intimate details of its activity.

Kinetic experiments[7,8] have shown that the enzyme–substrate, thio ester, complex is formed in essentially two phases. Actually, the dynamic, atomic interactions would obviously be much more intricate, owing to the variety of hydrophobic, ionic, and hydrogen-bonding regions available. The initial phase involves noncovalent binding (ES) of a substrate (S) to the enzyme (E). During this phase, the chief stereochemical preference is exhibited, often toward a choice of positions of attack within diastereoisomeric reactants, or else toward one isomer of a mixture of stereoisomers.[6,12,15] After the enzyme exerts this control, the main complexities of catalytic action of the second phase occur in two steps that require breakage and formation of covalent bonds in each step. The first step produces the thio ester (ES′) at the mercapto group of the enzyme,[7,8] while the second step[9] yields the principal reaction product (BS′). The acyl portion of the original substrate is thereby transferred to the enzyme and then to the reaction product. The progress of events can be expressed in the following abbreviated form[7,8,16] where B is the Lewis base.

$$E + S \rightleftarrows ES \longrightarrow ES' \xrightarrow{B} E + BS'$$

The substrate (S) must penetrate a crevice between the two lobes of the enzyme, where the mercapto group resides. The Lewis base can be water, during hydrolytic reactions,[12] an alcohol,[17] or an amino base such as aniline or substituted anilines,[6] which were employed in the current research. A generalized, tentative mechanism[18] is given in Scheme I.

Papain readily resolved *N*-(*tert*-alkoxycarbonyl)-DL-alanines. The percentage of L enantiomer in the product varied from ~95 to 100%, as listed in Table I.

TABLE I
PER CENT OF L ENANTIOMER IN THE PRODUCTS FORMED FROM SUBSTITUTED ANILINES AND *N*-(ALKOXYCARBONYL)-DL-ALANINES

Product	L Enantiomer, %
N-(*tert*-Butoxycarbonyl)alanine *p*-anisidide	95.3
N-(*tert*-Pentyloxycarbonyl)alanine *m*-anisidide	100.0
N-(*tert*-Pentyloxycarbonyl)alanine *p*-anisidide	99.3
N-(*tert*-Pentyloxycarbonyl)alanine *o*-fluoroanilide	99.7

Other details of these reactions are summarized in Table II. Space filled models[15b] have been used in conjunction with X-ray analysis of a bound, iodinated, competitive inhibitor, namely *N*-(*tert*-butoxycarbonyl)-L-*p*-iodophenylalanyl-L-leucine, as an excellent means for exposure of stereochemical control. Since the position of the iodine can be located, and since the carboxyl of this substrate must form a thio ester with the –SH of the enzyme, other features were easily inferred from the models. These included antiparallel, pleated sheet or β-structure types of interactions through peptide hydrogen bonding between the enzyme and this substrate. For racemic *N*-acylalanines, an L configuration is decidedly preferred by the enzyme because the α-CH of the substrate normally contacts the β-CH_2– of cysteine residue 25 of the enzyme. A concurrent proper disposition of the substrate carboxyl toward the –SH of cysteine residue 25 of papain and simultaneous hydrogen bonding at that carboxyl by the protonated imidazole ring of histidine residue 159 are necessary, as formulated in Scheme I. The α-CCH_3 of the D enantiomer largely inhibits such simultaneous contacts with the enzyme, thus explaining the stereochemical control exerted by the enzyme during resolutions of such substrates. From these well-chosen

(7) G. Lowe and A. Williams, *Proc. Chem. Soc. London*, 140 (1964).
(8) G. Lowe and A. Williams, *Biochem. J.*, **96**, 189, 199 (1965).
(9) J. L. Abernethy, L. Yengoyan, J. Seay, and J. Abu-Samra, *J. Org. Chem.*, **27**, 2528 (1962).
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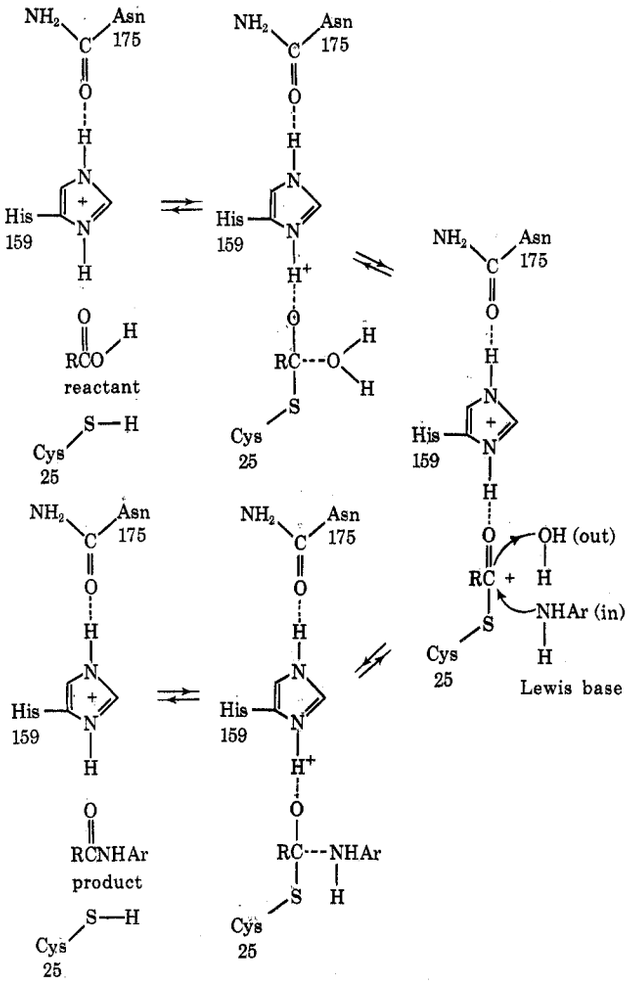
(14) J. Drenth, J. N. Jonsonius, R. Koekoek, and B. G. Wolthers, *Advan Protein Chem.*, **25**, 79 (1971).
(15) (a) A. Berger and I. Schechter, *Phil. Trans. Roy. Soc. London*, **B257**, 249 (1970); (b) A. Berger, I. Schechter, H. Benderly, and N. Kurn, *Proc. Eur. Symp. Peptide, 10th*, 290 (1971).
(16) J. R. Whitaker and M. L. Bender, *J. Amer. Chem. Soc.*, **87**, 2728 (1965).
(17) A. N. Glazer, *J. Biol. Chem.*, **241**, 3811 (1966).
(18) J. N. Drenth, J. N. Jansonius, R. Koekoek, L. A. A. Sluyterman, and B. G. Wolthers, *Phil. Trans. Roy. Soc. London*, **B257**, 231 (1970).

SCHEME I[a]

MECHANISM FOR PAPAIN CATALYSIS OF THE FORMATION OF ANILIDES OF *N*-ACYLAMINO ACIDS

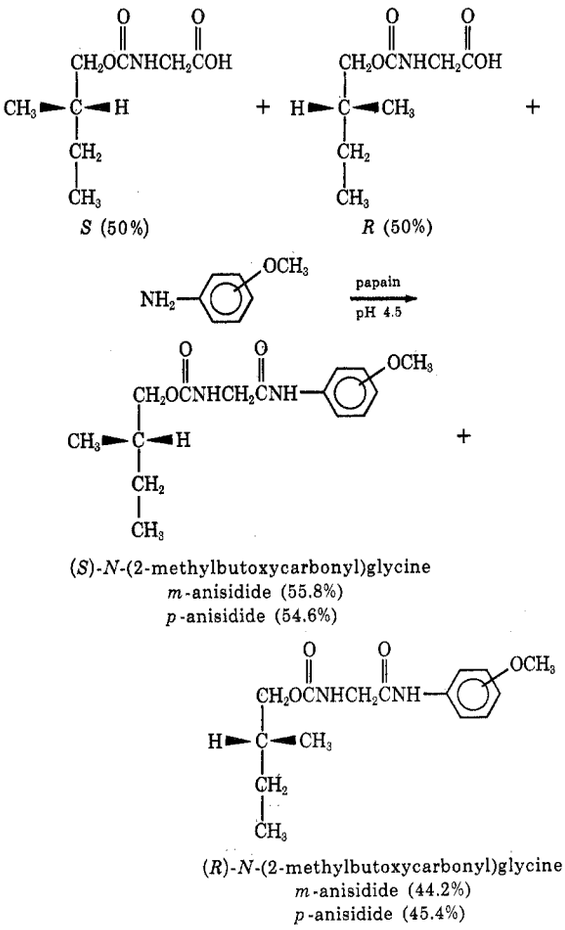


[a] More generalized terms: (a) in carbonyl-containing reactant, RC(=O)X, X can be –OH, –OR′, –NH$_2$, –NHR″ (a peptide or protein). (b) Lewis base, :B, can be :ÖH$_2$, :ÖHR, :NH$_2$Ar, :NH$_2$NHAr, :NH$_2$NHCOAr, :NH$_2$NHSO$_2$Ar.

experiments, much delineation of details has been possible. However, more intimate analyses of complex systems such as enzymes could certainly benefit through development of more revealing cybernetic approaches.

o-Anisidine and *o*-aminophenol did not give a reaction product, while *o*-fluoroaniline reacted very slowly. It would appear that an ortho substituent is sterically unfavorable. Reduction in the basicity of the amino group ordinarily decreases the reaction velocity of the substituted aniline owing to distortion of the nonbonding orbital in a manner that makes the electron pair less available for reaction. The same factors that increase the basicity of the amine, and therefore its reactivity in the unprotonated form, also decrease the concentration of the unprotonated amine added to the solution. Therefore the potential reaction velocity is reduced by formation of the conjugate acid at the acidic pH used for these experiments. The importance of this factor can readily be seen by inspection of the pK_a values for aniline, *o*-, *m*-, and *p*-anisidines, *o*-fluoroaniline, and *o*-aminophenol, which are respectively 4.6, 4.6, 4.2, 5.3, 3.2, and 4.8.[19] The concentration of unprotonated *o*-fluoroaniline would be more than 2 times as great as that of the unprotonated form of *o*-anisidine under comparable conditions of concentration of total base added at the same pH of 4.5.

In all instances, when (*R*,*S*)-*N*-(alkoxycarbonyl)-glycines were used as the reactants, at least moderate resolution was observed during the formation of the *m*-anisidides and *p*-anisidides. For (*R*,*S*)-*N*-(2-methylbutoxycarbonyl)glycine, the extent of resolution, as shown by specific rotation of the product in pyridine after a 3-hr incubation period, could be determined by replacement of the racemic reactant with (*S*)-*N*-(2-methylbutoxycarbonyl)glycine and then determination of the specific rotation of the *S* enantiomeric product. The percentage composition of the nonracemic products was calculated by the method previously reported.[20]



(*S*)-*N*-(2-methylbutoxycarbonyl)glycine
m-anisidide (55.8%)
p-anisidide (54.6%)

(*R*)-*N*-(2-methylbutoxycarbonyl)glycine
m-anisidide (44.2%)
p-anisidide (45.4%)

Papain is not able to distinguish as readily between enantiomers of these *N*-acylglycines when the asymmetric center is located in the *N*-alkoxycarbonyl group. Better recognition occurs when the asymmetric carbon is closer to the carboxyl group, as displayed by the experimental results. When the center is removed farther from the carboxyl, the control is probably chiefly through hydrophobic contact of the *N*-alkoxycarbonyl group with a hydrophobic region of the enzyme. Increased hydrolysis rates for esters of *N*-(benzyloxycarbonyl)amino acids, as compared with *N*-acetyl

(19) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution," Buttersworths, London, 1965.

(20) J. L. Abernethy, E. Albano, and J. Comyns, *J. Org. Chem.*, **36**, 1580 (1971).

TABLE II

PRODUCTS FROM N-(*tert*-ALKOXYCARBONYL)AMINO ACIDS AND SUBSTITUTED ANILINES FORMED BY PAPAIN-CATALYZED REACTIONS

Registry no.	Compd	Mp, °C	$[\alpha]^{25}D$ in Pyridine	N, %
34917-97-0	*N*-(*tert*-Butoxycarbonyl)glycine *m*-anisidide *t*-BOC-gly *m*-A[a]	109–110	Symmetric	Calcd: 9.99 Found: 9.65
34885-75-1	*N*-(*tert*-Butoxycarbonyl)glycine *p*-anisidide *t*-BOC-gly *p*-A	146–147	Symmetric	Calcd: 9.99 Found: 10.12
34885-76-2	*N*-(*tert*-Pentyloxycarbonyl)glycine *m*-anisidide *t*-POC-gly *m*-A	122–123	Symmetric	Calcd: 9.51 Found: 9.51
34885-77-3	*N*-(*tert*-Pentyloxycarbonyl)glycine *p*-anisidide *t*-POC-gly *p*-A	82–83	Symmetric	Calcd: 9.51 Found: 9.68
34885-78-4	*N*-(*tert*-Butoxycarbonyl)-L-alanine *m*-anisidide *t*-BOC-L-ala *m*-A	145–146	−67.9	Calcd: 9.51 Found: 9.38
	N-(*tert*-Butoxycarbonyl)alanine[b] *m*-anisidide *t*-BOC-ala *m*-A	144–146	−61.5	Mixture mp, no depression
34885-79-5	*N*-(*tert*-Pentyloxycarbonyl)-L-alanine *m*-anisidide *t*-POC-L-ala *m*-A	133–134	−63.5	Calcd: 9.08 Found: 8.90
	N-(*tert*-Pentyloxycarbonyl)alanine[b] *m*-anisidide *t*-POC-ala *m*-A	130–132	−63.5	Mixture mp, no depression
34885-80-8	*N*-(*tert*-Pentyloxycarbonyl)-L-alanine *p*-anisidide *t*-POC-L-ala *p*-A	102–104	−61.9	Calcd: 9.08 Found: 9.19
	N-(*tert*-Pentyloxycarbonyl)alanine[b] *p*-anisidide *t*-POC-ala *p*-A	102–103	−61.0	Calcd: 9.08 Found: 9.18
34885-81-9	*N*-(*tert*-Pentyloxycarbonyl)-L-alanine *o*-fluoroanilide *t*-POC-L-ala *o*-F	128–129	−68.4	Calcd: 9.45 Found: 9.66
	N-(*tert*-Pentyloxycarbonyl)alanine[b] *o*-fluoroanilide *t*-POC-ala *o*-F	125–126	−68.0	Mixture mp 125–126°

[a] Abbreviations used in the Experimental Section. [b] Products from *N*-acyl-DL-alanine often contain a small amount of the D enantiomer.

derivatives,[21] have been attributed to better hydrophobic bonding. Intimate details of hydrophobic bonding have been possible, by use of a molecular model, in connection with the previously mentioned iodinated inhibitor.[15b] For example, the α-CR side chain of the L-leucine residue, the iodinated phenyl group of the *p*-iodo-L-phenylalanyl residue, and the *N*-*tert*-butoxycarbonyl radical contact specific, but different, hydrophobic regions of the enzyme. Furthermore, insertion of an L-phenylalanyl residue into special polypeptides has shown that the phenyl group exerts powerful hydrophobic bonding with the enzyme, in such a manner that the enzyme attacks the polypeptide[15] at the carbonyl of a residue next to that phenylalanyl residue to form the thio ester intermediate, -SCOR, during hydrolysis. The R contains the remainder of the residue next to the phenylalanyl residue, and then the phenylalanyl residue in sequence.

The rigorous X-ray analysis of papain[14] immediately recognized the histidine residue 159 to be in juxtaposition across the dilobal region from the -SH group. The catalytic activity of the serine enzyme, α-chymotrypsin,[22] had been explained by a coordinated action between the serine residue -OH, which was acylated, and the imidazole ring of the histidine residue. There was hesitancy in the instance of papain to incorporate the protonated imidazole ring of histidine residue 159 because a nonenzymic imidazole ring of histidine did not have an appropriate pK_a value. When related to various other factors of the total primary structure and conformation of papain, the difficulty could be at least partially removed.[14] The present research has therefore been related to current information concerning papain. It has been conclusively demonstrated that the chirality of papain can bring about resolutions of racemic *N*-acylamino acids, even though the asymmetric center is at a considerable distance from the carboxyl group.

(21) G. Lowe, *Phil. Trans. Roy. Soc. London*, **B257**, 231 (1970).

(22) R. E. Dickerson and I. Geis, "The Structure and Action of Proteins," Harper and Row, New York, N. Y., 1969, p 83.

Experimental Section

Activation of Papain.—Papaya latex, imported from the Congo region of Africa, was extracted, activated, and dried as described previously,[20] with the exception that 400 ml of distilled water was used for each 100-g sample, rather than 100 ml.

***N*-Acylamino Acids.**—Many of the compounds are available from the Protein Research Foundation of Osaka University, Osaka, Japan. Dr. Shumpei Sakakibara, Director of this Foundation, cooperated in the preparation of *N*-(*tert*-pentyloxycarbonyl)-DL-alanine. Synthesis of this compound follows the general procedure which employs *tert*-pentyl chloroformate, formed from phosgene and *tert*-pentyl alcohol.[23] The ethyl ester was treated with *tert*-pentyl chloroformate in the presence of pyridine, followed by saponification[24] to the *N*-acylamino acid. *N*-(*tert*-Butoxycarbonyl)-DL-alanine was prepared[25] by the Fox Chemical Co. of Los Angeles. These data are pertinent. *N*-(*tert*-Pentyloxycarbonyl)-DL-alanine had mp 105–106°. *Anal.* Calcd: N, 6.89. Found: N, 6.86. *N*-(*tert*-Butoxycarbonyl-DL-alanine had mp 111–112°. *Anal.* Calcd: N, 7.40. Found: N, 7.50.

The four *N*-(alkoxycarbonyl)glycines were synthesized in cooperation with Dr. Shumpei Sakakibara, starting with (*R,S*)-2-butanol, (*R,S*)-2-pentanol, (*R,S*)-2-methyl-1-butanol, and (*S*)-2-methyl-1-butanol, $[\alpha]^{20}D$ −4.5° (neat). The alkyl chloroformates of these alcohols,[26–29] were prepared with excess liquid

(23) (a) R. Schyzer, P. Sieber, and K. Kappeler, *Helv. Chim. Acta*, **42**, 2622 (1952); (b) E. Schnabel, *Justus Liebigs Ann. Chem.*, **702**, 188 (1960).

(24) S. Sakakibara, M. Shin, M. Fujino, Y. Shinonishi, S. Inoye, and N. Inuka, *Bull. Chem. Soc. Jap.*, **38**, 1522 (1965).

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(26) K. H. Meyer and Y. Go., *Helv. Chim. Acta*, **17**, 1488 (1934).

(27) F. C. McKay and N. F. Albertson, *J. Amer. Chem. Soc.*, **79**, 4686 (1957).

(28) A. C. Chibnal and P. F. Spahr, *Biochem. J.*, **68**, 135 (1958).

(29) S. Sakakibara, Director, Protein Research Foundation, Osaka University, Osaka, Japan, private communications (1970).

phosgene, in the cold, with subsequent removal of excess phosgene under reduced pressure, and were then treated with glycine[26,28] or ethyl glycinate.[24] The product from glycine was acidified with a slight excess of sulfuric acid, extracted into ethyl acetate, dried over anhydrous sodium sulfate, and evaporated at low temperature. The product from ethyl glycinate was first saponified and then worked up similarly. Since these *N*-acylamino acids were oils, they were converted into the dicyclohexylammonium salts by reaction with dicyclohexylamine dissolved in ether.[24] These follow with their properties: (*R,S*)-*N*-(*sec*-butoxycarbonyl)-glycine DCHA, mp 128–129°, % N calcd 7.86 and found 7.55; (*R,S*)-*N*-(1-methylbutoxycarbonyl)glycine DCHA, mp 121.5–123°, % N calcd 7.56 and found 7.70; (*R,S*)-*N*-(2-methylbutoxycarbonyl)glycine DCHA, mp 121–122°, % N calcd 7.56 and found 7.31; (*S*)-*N*-(2-methylbutoxycarbonyl)glycine DCHA, mp 118–119.5°, % N calcd 7.56 and found 7.51.

Isolation of *N*-(Alkoxycarbonyl)glycines from Their Dicyclohexylammonium Salts.—The method was a modification of the directions outlined by the Protein Research Institute of Osaka University, Osaka, Japan. Ten grams of the powdered salt was placed in a separatory funnel with 100 ml of ethyl acetate and shaken for several minutes. Then, for each equivalent of salt there was added 1.2 equiv of 1 *N* H_2SO_4. The mixture was shaken until all of the salt had dissolved. The ethyl acetate layer was separated and the aqueous layer was extracted three more times, with the same volume of ethyl acetate each time. The combined ethyl acetate extracts were then washed once with about 25 ml of a saturated sodium chloride solution. Suction filtration was necessary to remove a small amount of insoluble precipitate. After separation of the ethyl acetate layer, it was dried over anhydrous sodium sulfate. The first addition of anhydrous salt produced an aqueous layer in contact with hydrated salt. Suction filtration of the entire mass was followed by separation of the ethyl acetate layer from the aqueous layer. More anhydrous sodium sulfate was added to the ethyl acetate layer and the mixture was shaken once more. The solid was removed by filtration and the ethyl acetate filtrate was placed over anhydrous sodium sulfate. The solid residue from these last two filtrations were extracted with about 50 ml of ethyl acetate and each extract was removed by filtration. These combined filtrates were added to the ethyl acetate that had been placed over anhydrous sodium sulfate. The mixture was shaken for several minutes and allowed to stand overnight. The solution was then removed by suction filtration. The solid was extracted with 30 ml of ethyl acetate and the ethyl acetate was removed by filtration and combined with the dried ethyl acetate layer. Flash evaporation was carried out from a 100-ml flask that was rapidly rotated. More solution was added from time to time. The flask, which contained two small pieces of glass rod to prevent bumping, had been previously weighed. Evaporation was continued to constant weight. A nearly quantitative yield of *N*-acylamino acid resulted as an oil. It was used directly, by dissolving in buffer, for the papain-catalyzed reactions. This isolation was also used with *N*-(*tert*-pentyloxycarbonyl)-L-alanine DCHA, purchased from Osaka University Protein Research Foundation.

General Procedures for Papain-Catalyzed Reactions.—Nearly all reaction mixtures contained 0.01 mol of the *N*-acylamino acid, 0.0100 mol of the substituted aniline, 0.500 g of L-cysteine·HCl·H_2O, 0.500 g of activated papain, and 100 ml of buffer (0.50 *M* HOAc), pH 4.5, placed in a 125-ml glass-stoppered flask. Incubation was started immediately at 40°. Insoluble products were removed by filtration at the end of appropriate periods of time, after which incubation was continued. In the cases of *N*-(*tert*-pentyloxycarbonyl)glycine *p*-anisidide and *N*-(*tert*-butoxycarbonyl)glycine *m*-anisidide it was necessary to induce crystallization by stirring vigorously in an ice bath after the first period of incubation, to change the oily product to a solid before filtration. Subsequent incubation yielded beautifully crystalline products without such treatment. For the reactions between *N*-(*tert*-pentyloxycarbonyl)-DL-alanine and *o*-fluoroaniline, *m*-anisidine, and *p*-anisidine, 0.0200 mol of the *N*-acylamino acid was used rather than 0.0100 mol. The other details were identical. Results for *N*-(*tert*-alkoxycarbonyl)amino acids are summarized in Table II. *o*-Anisidine, *o*-aminophenol, and *o*-fluoroaniline failed to give satisfactory reactions when substituted glycines were used.

Weights of Precipitates from Reaction between *N*-(*tert*-Alkoxycarbonyl)alanines and Substituted Anilines.—Precipitates were collected by suction filtration after various periods of incubation. The filtrate was returned to the incubator. Then the precipitate was washed, on the suction filter, with about 200 ml of distilled water. The solid, on the filter paper, was removed and dried near the vent of a hood with the hood turned on, for a period of 24 hr and then weighed. Purification for nitrogen analyses and for optical rotations was accomplished by dissolving the precipitates in methanol, adding a small amount of decolorizing carbon, and filtering four times, with careful rinsing with methanol each time to remove the solid completely. For the racemic *N*-acylamino acids, only the products from the 0–24 hr periods of incubation were used for purification. Carefully cleaned filter flasks and Buchner funnels were used for each repetitive filtration. The final filtrate was placed in a Petri dish with a slightly elevated watch glass and dried by evaporation under the hood. This was followed by drying in a vacuum desiccator over phosphorus pentoxide.

Three groups of weights are given, A, B, and C. For the A group, incubation periods in sequence were 0–24 and 24–48 hr; for the B group 0–24 hr only; for the C group 0–6, 6–12, 12–24, and 24–48 hr. Abbreviations for names of precipitates are those given in Table II. Group A. *t*-BOC-gly *m*-A: 1.162 g, 0.057 g; *t*-BOC-gly *p*-A: 1.752 g, 0.397 g; *t*-POC-gly *m*-A: 1.438 g, 0.188 g; *t*-POC-gly *p*-A: 1.157 g, 0.056 g. Group B. *t*-BOC-L-ala *p*-A: 0.882 g; *t*-BOC-ala *p*-A: 1.350 g. Group C. *t*-POC-L-ala *m*-A: 1.584 g, 0.092 g, 0.197 g, 0.186 g; *t*-POC-ala *m*-A: 2.225 g, 0.474 g, 0.219 g, 0.093 g; *t*-POC-L-ala *p*-A: 1.674 g, 0.178 g, 0.089 g, 0.031 g; *t*-POC-ala *p*-A: 1.325 g, 0.456 g, 0.266 g, 0.125 g; *t*-POC-L-ala *o*-F: 0.226 g, 0.186 g, 0.179 g, 0.178 g; *t*-POC-ala *o*-F: 0.108 g, 0.032 g, 0.027 g, 0.017 g.

Optical Rotations.—For optical rotations of products from *N*-(*tert*-alkoxycarbonyl)-DL- or -L-alanine, Eastman Spectro Grade pyridine was used, with a Rudolph Model 80 high precision polarimeter. Optical rotations of products from (*R,S*)-*N*-(alkoxycarbonyl)glycines, unless otherwise specified, were determined for purified products from the earliest incubation period in spectro grade pyridine at 25° (room temperature) and 589 nm with a Cary Model 60 recording spectropolarimeter at the University of California, Los Angeles. Zero settings were made for each measurement before and after the optical measurement was made, with pyridine in the polarimeter tube of 1-cm thickness. Solids were dissolved in pyridine in a 5.00-ml volumetic flask and the solutions were then filtered through sintered-glass suction tubes. After a zero reading with the solvent, the same polarimeter tube was rinsed with methanol and then ether and then dried with the use of a constricted glass tubing connected to a suction line. The polarimeter tube was filled with solution and optical rotation was taken. For a low optical rotation, a setting was used for 100 divisions/0.02°. For other rotations, a setting of 100 divisions/0.04° was used. Solutions involved ~0.1 to 0.6 g of *N*-acylamino acid/5 ml of solution. All were weighed to the nearest 0.1 mg.

Systematic Recording of Experimental Results for Reactions between *N*-(Alkoxycarbonyl)glycines and *o*- and *p*-Anisidines.—At the beginning of each incubation period, there would be equal quantities of *R* and *S* enantiomers when a racemic *N*-acylamino acid was used. However, their anisidide products would not ordinarily contain equal amounts of *R* and *S* enantiomers. Therefore, these products are designated as *R* and *S*, rather than *R,S*. For products from the essentially single (*S*)-*N*-acylamino acid, the symbol *S* is used. Hours of incubation are listed first, followed by the weight of product, and then the melting point. Optical rotations and nitrogen analyses are given at the end. A single asterisk (*) indicates that the purified product from that incubation period was used for both an optical rotation in pyridine as the solvent and for nitrogen analysis. A double asterisk (**) means that the purified product was used only for a nitrogen analysis, a triple asterisk (***) only for optical rotation.

I. (*R*)- **and** (*S*)-*N*-(*sec*-**Butoxycarbonyl)glycine** *m*-**anisidides:** 0–3 hr, 0.261 g*, 75–77°; 3–6 hr, 0.062 g, 76–77°; 6–12 hr, 0.002 g, 76–77°; 12–24 hr, 0.009 g, 74–75°; 24–48 hr, 0.005 g, 74–75° ($[\alpha]^{25}D$ −0.11°, % N calcd 9.99 and found 9.94). (*R*)- **and** (*S*)-*N*-(*sec*-**Butoxycarbonyl)glycine** *p*-**anisidides:** 0–3 hr, 0.140 g***, 117–119°; 3–6 hr, 0.243 g, 119–120°; 6–12 hr, 0.163 g**, 119–120°; 12–24 hr, 0.064 g, 119–120°; ($[\alpha]^{25}D$ −0.97°, % N calcd 9.99 and found 9.90).

II. (*R*)- **and** (*S*)-*N*-(1-**Methylbutoxycarbonyl)glycine** *m*-**anisidides:** 0–6 hr, 1.184 g*, 116–117°; 6–12 hr, 0.185 g, 119–120°; 12–24 hr, 0.179 g, 117–118°; 24–48 hr, 0.107 g, 116–118°; ($[\alpha]^{25}D$ +1.44°, % N calcd 9.51 and found 9.52).

III. (*S*)-*N*-(**2-Methylbutoxycarbonyl**)**glycine** *m*-**anisidide:** 0–6 hr, 1.190 g*, 102–103°; 6–12 hr, 0.117 g, 102–103°; 12–24 hr, 0.024 g 101–102°; ($[\alpha]^{25}D$ +1.20°, % N calcd 9.51 and found 9.62). (*R*)- **and** (*S*)-*N*-(**2-Methylbutoxycarbonyl**)**glycine** *m*-**anisidides:** 0–3 hr, 1.037 g*, 78–79°; 3–6 hr, 0.353 g, 76–77°; 6–12 hr, 0.199 g, 76–77°; 12–24 hr, 0.061 g, 76–77°; ($[\alpha]^{25}D$ +0.138°, % N calcd 9.51 and found 9.52, 55.8% *S* enantiomer in 0–3-hr product.

IV. (*S*)-*N*-(**2-Methylbutoxycarbonyl**)**glycine** *p*-**anisidide:** 0–6 hr, 1.168 g*, 116–118°; 6–12 hr, 0.346 g, 116–118°; 12–24 hr, 0.132 g, 116–118°; ($[\alpha]^{25}D$ +2.40°, % N calcd 9.51 and found 9.70). (*R*)- **and** (*S*)-*N*-(**2-Methylbutoxycarbonyl**)**glycine** *p*-**anisidides:** 0-3 hr, 0.856 g*, 111–112°; 3–6 hr, 0.456 g, 111–112°; 6–12 hr, 0.397 g, 110–111°; 12–24 hr, 0.174 g, 110–111°; 24–48 hr, 0.130 g, 107–109°; ($[\alpha]^{25}D$ +0.222°, % N calcd 9.51 and found 9.70, 54.6% *S* enantiomer in 0–3 hr product).

Registry No.—*N*-(*tert*-Butoxycarbonyl)glycine, 4530-20-5; *N*-(*tert*-pentyloxycarbonyl)glycine, 3588-44-1; *N*-(*tert*-butoxycarbonyl)-DL-alanine, 3744-87-4; *N*-(*tert*-pentyloxycarbonyl)-DL-alanine, 34885-82-0; (*R,S*)-*N*-(*sec*-butoxycarbonyl)glycine DCHA, 38435-97-1; (*R,S*)-*N*-(1-methylbutoxycarbonyl)glycine DCHA, 38435-98-2; (*R,S*)-*N*-(2-methylbutoxycarbonyl)glycine DCHA, 38435-99-3; (*S*)-*N*-(2-methylbutoxycarbonyl)glycine DCHA, 38436-00-9; (*R*)-*N*-(*sec*-butoxycarbonyl)glycine *m*-anisidide, 38436-01-0; (*S*)-*N*-(*sec*-butoxycarbonyl)glycine *m*-anisidide, 38436-02-1; (*R*)-*N*-(*sec*-butoxycarbonyl)glycine *p*-anisidide, 38436-03-2; (*S*)-*N*-(*sec*-butoxycarbonyl)glycine *p*-anisidide, 38436-04-3; (*R*)-*N*-(1-methylbutoxycarbonyl)glycine *m*-anisidide, 38436-05-4; (*S*)-*N*-(1-methylbutoxycarbonyl)glycine *m*-anisidide, 38436-06-5; (*S*)-*N*-(2-methylbutoxycarbonyl)glycine *m*-anisidide, 38436-07-6; (*R*)-*N*-(2-methylbutoxycarbonyl)glycine *m*-anisidide, 38436-08-7; (*S*)-*N*-(2-methylbutoxycarbonyl)glycine *p*-anisidide, 38436-09-8; (*R*)-*N*-(2-methylbutoxycarbonyl)glycine *p*-anisidide, 38436-10-1; papain, 9001-73-4.

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Purine *N*-Oxides. XLVI. Some Interesting Reactions of 3-Acetoxy-8-methylxanthine[1]

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The reactivities of 3-acetoxy-8-methylxanthine have been compared with corresponding reactivities of 3-acetoxyxanthine. The former undergoes a rearrangement in water to yield 8-hydroxymethylxanthine, and evidence is presented suggesting an intermediate possessing an exocyclic methylene group. In addition, some hydrolysis to 3-hydroxy-8-methylxanthine and some reduction to 8-methylxanthene occur, the latter apparently proceeding through a radical mechanism. 8-Methylguanine 3-oxide can also be rearranged to 8-hydroxymethylguanine. 3-Acetoxyxanthine reacts in aqueous solutions with many nucleophiles to yield a variety of 8-substituted xanthines. Under the same conditions 3-acetoxy-8-methylxanthine reacts only with the water to afford the 8-hydroxymethyl derivative. 3-Acetoxy-8-azaxanthine undergoes only the hydrolysis and reduction reactions.

Esters of the oncogenic 3-hydroxyxanthine react with nucleophiles under mild conditions *in vitro*,[3] and *in vivo*,[4] to form 8-substituted xanthines. Similar nucleophilic substitution reactions with macromolecules of the cell have long been offered as an explanation of the initiation of the cancer process by chemical oncogens.[5] A weak oncogenicity of 3-hydroxy-8-azaxanthine (**16**) and the possible similar activity of 3-hydroxy-8-methylxanthine (**1**)[4] prompted a comparative investigation of the chemical behavior of compounds with these distinct alterations of the 8 position of 3-hydroxyxanthine.

Treatment of 3-hydroxy-8-methylxanthine (**1**) with acetic anhydride in trifluoroacetic acid at room temperature afforded a monoacetyl derivative. The ir and nmr[6] spectra indicated the presence of an O-acetyl group, and the slow development of a purple color with ferric chloride provided further support for the 3-acetoxy-8-methylxanthine structure (**2**). Reaction of 3-hydroxy-8-methylxanthine (**1**) in hot acetic anhydride, followed by treatment with water, gave some 8-hydroxymethylxanthine (**10**) and extensive decomposition. The *O*-acetyl derivative of 3-hydroxy-8-methylguanine (**14**) was not isolable, but in acetic anhydride and trifluoroacetic acid at room temperature, followed

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(2) D. R. S. is a Damon Runyon Memorial Fellow.

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(6) A downfield shift of the nmr signal of the 8-methyl protons in 8-methylxanthine and guanine derivatives, when the solvent was TFA rather than DMSO-d_6, was attributed to protonation of the imidazole ring in the former solvent. This phenomenon has been observed previously in N-methylated xanthines.[7]

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