CCLXXI. ENZYMIC PROTEOLYSIS

III. HYDROLYSIS OF ASPARAGINE, ASPARAGINE PEPTIDES AND ANHYDROGLYCYL-ASPARAGINE

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THE experiments to be described in the present paper are complementary to those published in a previous communication [Damodaran & Ananta-Narayanan, 1938] on the formation of ammonia during the enzymic hydrolysis of natural proteins. Bergell & Wulfig [1910], who seem to have been the first to study the deamidizing action of proteolytic enzymes on synthetic substrates, showed that *dl*-leucinamide is hydrolysed by pancreatin, the *l*-compound being more readily decomposed than the *d*-isomeride; negative results were recorded in the case of glycineamide, alanineamide and natural asparagine. According to Levene *et al.* [1926] "filtered intestinal juice" splits glycineamide as readily as alanylalanine though not with the same ease as glycylglycine. Grover & Chibnall [1927] observed the hydrolysis of asparagine by intestinal erepsin as well as by a dipeptidase preparation obtained from germinating barley. The hydrolysis of glycineamide and leucinamide was confirmed by Waldschmidt-Leitz *et al.* [1927] using trypsin-free erepsin prepared by adsorption. Melville [1935] attempted to study the deamidizing action of proteolytic enzymes on γ -glutamyl peptides, but found that the substrates were so labile that they were spontaneously decomposed in aqueous solutions at the *pH* required for enzyme action.

In the present investigation we have studied the actions of pepsin, trypsin, erepsin and papain on asparagine, glycineamide, *dl*-leucinamide, glycyl-*l*-asparagine, *d*- and *l*-leucylasparagine, chloroacetyl-*l*-asparagine and anhydroglycylasparagine (2:5-diketopiperazine acetamide). Amide hydrolysis was measured by actual distillation of the ammonia and estimation of the latter by a sensitive micro-method. To ensure that quantitative differences in the rate of splitting were not interpreted to mean qualitative differences in specificity, enzyme action was in all cases allowed to proceed for fairly long periods. Some of the substrates, particularly glycineamide, glycyl- and leucyl-asparagine, were found to be hydrolysed to a slight extent at the *pH* employed for enzyme action, but rigorous controls were run to determine the exact extent of such spontaneous decomposition. The results are summarized in Table I.

Pepsin and purified trypsin have no action on either the peptide or amide bonds in any of the substrates examined. Trypsin-free erepsin on the other hand has a definite, though slow, deamidizing action on asparagine, the hydrolysis of the amide group proceeding more rapidly in fact than in glycineamide though less rapidly than in leucinamide. The amide group in asparagine peptides does not appear to be directly hydrolysed; a small amount of ammonia is formed from glycylasparagine and *l*-leucyl-*l*-asparagine, but this must depend on the preliminary hydrolysis of the peptide to give free asparagine as no ammonia liberation takes place from *d*-leucylasparagine in which the peptide bond is not split by erepsin. Of particular interest in connexion with peptide hydrolysis is the finding that in chloroacetyl-asparagine the peptide bond is split by erepsin in

Table I. *Hydrolysis %*

	Trypsin-free erepsin		Erepsin-free trypsin		Pepsin		Papain-HCN	
	Peptide	Amide	Peptide	Amide	Peptide	Amide	Peptide	Amide
<i>l</i> -Asparagine	—	10 15 days	—	0.37 7 days	—	0.43 6 days	—	0.33 9 days
Glycinamide	—	10 10 days	—	Nil	—	2.9 9 days	—	1.45 8 days
<i>dl</i> -Leucinamide	—	53 10 days	—	1.0 7 days	—	Nil	—	11.0 7 days
Glycyl- <i>l</i> -asparagine	97 10 days	8 26 days	Nil	Nil	Nil	Nil	Nil	Nil
<i>d</i> - and <i>l</i> -Leucyl- <i>l</i> -asparagine	17 6 days	2 6 days	„	„	„	„	„	„
<i>d</i> -Leucyl- <i>l</i> -asparagine	Nil	Nil	„	„	„	„	„	„
Chloroacetyl- <i>l</i> -asparagine	77 10 days	10 10 days	„	„	„	„	„	„
Anhydroglycylasparagine	Nil	Nil	„	„	„	„	„	„

spite of the absence of a free amino group adjacent to it; this is analogous with the hydrolysis by this enzyme of chloroacetylalanine observed by Abderhalden & Ehrenwall [1930]. According to the views on enzyme specificity developed by Waldschmidt-Leitz, Grassmann and collaborators (summaries by Grassmann [1928, 1932, 1936]) trypsin-free erepsin consists of a mixture of aminopolypeptidase and dipeptidase. The observations now recorded can be brought into line with the views on enzyme specificity developed by the Willstätter school only on the assumption that, in addition to di- and aminopoly-peptidases, erepsin also contains asparaginase and other amidases as well as peptidases that act specifically on acyl peptides of the type of chloroacetyl-asparagine. However, the weight of available evidence is against the acceptance of these views of the mode of action of the proteases as final. On the one hand the identification of some of the proteolytic enzymes with crystalline proteins [Northrop, 1930; Northrop & Kunitz, 1934], the changes in specificity shown to take place on keeping of enzymes purified by adsorption [Abderhalden & Schmitz, 1929; Abderhalden & Ehrenwall, 1931] and the dependence of the range of activity of proteases on physical conditions [Fodor, 1930] make it highly improbable that the enzyme preparations with restricted activity obtained by Waldschmidt-Leitz, Grassmann and others really represent individual enzymes in the natural state and not merely fragments of such. Further, numerous recent studies on synthetic peptides, in particular by Abderhalden and by Bergmann, make it clear that the chemical bases of the specificity relations enunciated by the Willstätter school for their enzyme preparations will have to be considerably modified.

With papain-HCN only a slow deamidation with leucinamide and hardly any with other substrates was noticed. This appears to be in contradiction with the results obtained previously [Damodaran & Ananta-Narayanan, 1938] which showed that papain digestion led to the formation of considerable amounts of ammonia. It has, however, been recently shown by Bergmann *et al.* [1935] that the amide group in peptide amides in which there is no free amino group in the proximity of the amide group is attacked by papain.

The observation that anhydroglycylasparagine is not hydrolysed either at the peptide or the amide bond by any of the enzymes tried is contrary to the claims

of Japanese workers [cf. Shibata, 1934] who claim to have demonstrated the enzymic hydrolysis of diketopiperazines, and provides further confirmation of the findings of Waldschmidt-Leitz & Gärtner [1936] with diketopiperazine acetic and propionic acids and of Greenstein [1936] with lysylglutamic acid anhydride.

With reference to the problem in protein structure which primarily prompted this investigation, the results obtained, taken in conjunction with previous findings [Damodaran, 1932; Damodaran *et al.* 1932; Melville, 1935; Damodaran & Ananta-Narayanan, 1938], agree with the assumption that the amide groups attached to the dicarboxylic amino-acids are the only important precursors of the ammonia formed in the acid hydrolysis of proteins. In the last paper cited above it was concluded that the ammonia liberated during the digestion of proteins by pepsin, trypsin and erepsin in succession was not primarily connected with enzyme action. If asparagine and glutamine are the only ammonia-yielding complexes in proteins this is readily understandable for the following reasons. Peptides of glutamine formed through its free (γ) carboxyl group are, unlike those formed through the amino group studied by Thierfelder & Cramm [1919], as unstable as glutamine and spontaneously decompose in aqueous solution [Melville, 1935] giving rise to ammonia. This non-enzymic ammonia formation could commence practically at the very beginning of peptic digestion and would increase progressively with the amount of glutamine-containing polypeptides set free. Asparagine peptides on the other hand are quite stable in aqueous solution and also, as shown in this paper, not acted upon by either pepsin, trypsin or erepsin. The deamidizing action which erepsin has been shown to have on *free* asparagine can come into play only when digestion is practically complete and is at the same time too slow to cause any appreciable liberation of ammonia. When it is further considered that the proportion of aspartic acid in proteins is invariably much lower than that of glutamic acid it is obvious that practically the whole of the ammonia formed during digestion has its origin in the secondary non-enzymic decomposition of glutamine peptides and of glutamine.

EXPERIMENTAL

Substrates

Glycinamide hydrochloride and *dl-leucinamide* hydrobromide were prepared according to Bergell & Wulfig [1910].

Glycyl-l-asparagine. The purified peptide prepared by the method of Fischer & Koenigs [1904] browned at 215° and gave the reactions described by these authors. N found 22.07 %; calc. for $C_6H_{11}N_3O_4$, 22.22 %. Amino-N (Van Slyke) was 124 % of the theoretical, in keeping with the observed abnormality of glycine and glycyl polypeptides in general [Van Slyke, 1911; Fischer & Koelker, 1910].

Leucyl-l-asparagine [Fischer & Koenigs, 1907]. Asparagine was coupled with bromoisohexoylbromide and the resulting compound treated with 75 % ammonia at room temperature for 6 days. Owing to the difference in solubility between the two isomeric bromisocapronylasparagines noted by Fischer & Koenigs, two products were finally obtained, viz. pure *d-leucyl-l-asparagine* and a mixture of *d-* and *l-leucyl-l-asparagines* in which the compound containing the non-natural *d-leucyl* residue predominated. Attempts to obtain the pure *l-leucyl*peptide in pure condition by fractional crystallization were not successful. $C_{10}H_{19}N_3O_4 \cdot 2H_2O$ requires N, 14.95 %; N, found, 15.2 % (for the *d* form) and 15.12 % (for the mixture of *d-* and *l-*).

Anhydroglycylasparagine. This was prepared according to Fischer & Koenigs [1904].

Enzymes

Erepsin-free trypsin [Waldschmidt-Leitz & Harteneck, 1925], trypsin-free erepsin [Waldschmidt-Leitz & Schäffner, 1926] and B.D.H. pepsin were used. Papain was prepared as previously described [Damodaran & Ananta-Narayanan, 1938].

Buffer solutions

For erepsin studies *M*/5 phosphate buffer at *pH* 7·8, for trypsin *M*/5 veronal buffer at *pH* 8·4 and for papain *M*/10 citrate buffer at *pH* 5 were employed. As the usual $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$ buffer for tryptic studies could not be used on account of the necessity of making ammonia determinations, experiments were conducted to ascertain the suitability of veronal buffer. It was found that veronal could effectively replace phosphate as well as $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$ in erepsin and trypsin digests. It has the advantage over the former of not interfering with titrations in alcohol or formalin.

Methods

Weighed amounts of the analytically pure substrates were dissolved in water, neutralized with a few drops of NaOH and, after the addition of buffer solution and enzyme, made up to give usually *M*/10 solutions. A few drops of toluene were added as antiseptic.

Peptide hydrolysis was determined by the usual alcoholic titration and ammonia liberation according to Parnas & Heller [1924] with the modification that when the ammonia to be determined was small titration was carried out by the iodimetric method of Henriques & Christiansen [1916] using *N*/280 acid.

(1) *Asparagine*Table II. *Exp. 1. Trypsin-free erepsin on l-asparagine*

Digest: *M*/10 asparagine containing 10 ml. of erepsin solution in total volume 25 ml. at *pH* 7·8 in phosphate buffer.

Time	Digest	Ammonia as ml. <i>N</i> /280 acid in 2 ml.			Amide hydrolysis %
		Substrate control	Enzyme control	Digest (corrected)	
0	0·04	Nil	0·04	Nil	Nil
6 hr.	0·20	Nil	0·03	0·17	0·32
18 hr.	0·79	Nil	0·03	0·76	1·36
42 hr.	1·92	0·02	0·06	1·85	3·30
4 days	4·00	0·09	0·08	3·82	6·80
10 days	8·78	0·18	0·08	8·52	15·20
19 days	12·16	0·34	0·13	11·65	20·80

Exp. 2. Erepsin-free trypsin on l-asparagine. Digest: *M*/10 asparagine containing 5 ml. of trypsin solution in total volume 25 ml. at *pH* 8·4 (veronal buffer).

Amide hydrolysis in 7 days, 0·37 %.

Exp. 3. B.D.H. pepsin on l-asparagine. Digest: 10 ml. of *M*/4 asparagine + 5 ml. pepsin solution in *N*/20 HCl containing 1/20 the weight of dry pepsin calculated on the weight of the substrate + 5 ml. *N*/5 HCl. Total volume 25 ml.

Amide hydrolysis in 6 days, 0·43 %.

Exp. 4. Papain-HCN on l-asparagine. Digest: 10 ml. *M*/4 asparagine. 5 ml. papain solution 10 ml. citrate buffer *pH* 5 in total volume 25 ml. No NH_3 in substrate control.

Amide hydrolysis in 9 days, 0·33 %.

(2) *Glycinamide*Table III. *Exp. 5. Trypsin-free erepsin on glycinamide*

Digest: *M*/12 glycinamide containing 10 ml. erepsin solution in total volume 25 ml. at pH 7.8 in phosphate buffer.

Time	Digest	Ammonia as ml. <i>N</i> /280 acid in 1 ml.			Amide hydrolysis %
		Substrate control	Enzyme control	Digest (corrected)	
0	0.16	0.12	0.04	0	0
5 hr.	0.26	0.12	0.04	0.10	0.43
21 hr.	0.55	0.25	0.03	0.27	1.16
46 hr.	0.93	0.35	0.08	0.50	2.15
4 days	1.65	0.59	0.06	1.00	4.29
10 days	3.11	0.70	0.08	2.33	10.00
19 days	4.48	1.69	0.11	2.68	11.50

Exp. 6. Erepsin-free trypsin on glycinamide. Digest: 0.3467 g. glycinamide HCl dissolved in water, neutralized with NaOH and made up to 25 ml. 10 ml. of this solution + 5 ml. veronal buffer at pH 8.4 + 5 ml. trypsin solution + water to 25 ml.

Amide hydrolysis in 7 days, nil.

Exp. 7. Pepsin on glycinamide. Digest: 0.1906 g. glycinamide HCl dissolved in sufficient HCl so that the final concentration of the HCl was *N*/20. 5 ml. of this solution removed and made up to 6.5 ml. for substrate control. To the residual 20 ml. 5 ml. pepsin solution in *N*/20 HCl added.

Amide hydrolysis, 1.1 % in 2 days, 1.9 % in 9 days.

Exp. 8. HCN-activated papain on glycinamide. Digest: 0.2241 g. glycinamide HCl + 10 ml. citrate buffer, pH 5, to total volume 25 ml. 5 ml. removed for substrate control. Residual 20 ml. + 5 ml. enzyme formed the digest.

Amide hydrolysis, 0.85 % in 65 hr., 1.45 % in 8 days.

(3) *dl-Leucinamide*Table IV. *Exp. 9. Trypsin-free erepsin on dl-leucinamide*

Digest: 0.3517 g. *dl*-leucinamide HBr made up to 25 ml. after neutralization and addition of *M*/5 phosphate buffer pH 7.8. 10 ml. of this solution + 5 ml. erepsin made up to 25 ml. with water.

Time	Digest	Ammonia as ml. <i>N</i> /280 acid in 2 ml.			Amide hydrolysis %
		Substrate control	Enzyme control	Digest (corrected)	
0- $\frac{1}{2}$ hr.	0.41	0.19	0	0.22	1.47
6 hr.	2.05	—	0	1.86	12.45
7 hr.	2.14	—	0	1.95	13.05
14 hr.	3.74	—	0	3.55	23.75
24 hr.	5.54	—	0	5.35	35.81
40 hr.	6.05	0.11	0	5.94	39.76
10 days	8.05	0.14	0	7.91	52.94
19 days	7.99	0.17	0	7.84	52.34

Exp. 10. Erepsin-free trypsin on dl-leucinamide. Digest: 0.175 g. *dl*-leucinamide HBr dissolved in water, neutralized and made up to 25 ml. with veronal buffer pH 8.4. 5 ml. of this removed for substrate control. The residual 20 ml. + 5 ml. trypsin formed the digest.

Amide hydrolysis in 7 days, 0.70 %.

Exp. 11. Pepsin on dl-leucinamide. Digest: 0.236 g. *dl*-leucinamide HBr dissolved in 25 ml. 5 ml. removed for substrate control. Residual 20 ml. + 5 ml. pepsin formed the digest.

Amide hydrolysis in 8 days, nil.

Table V. *Exp. 12. Papain-HCN on dl-leucinamide*

Digest: 0.1902 g. *dl*-leucinamide HBr at pH 5 containing 5 ml. papain in total volume 25 ml.

Time	Ammonia as ml. <i>N</i> /280 acid in 2 ml.				Amide hydrolysis %
	Digest	Substrate control	Enzyme control	Digest (corrected)	
0	2.04	0	2.04	0	0
4 hr.	1.94	0	—	0	—
16 hr.	2.02	0	—	0	—
25 hr.	2.23	0	—	0.19	0.94
51 hr.	2.71	0	—	0.67	3.30
4 days	4.01	0	—	1.97	9.70
7 days	4.25	0	—	2.21	10.90

(4) *Glycyl-L-asparagine*

Table VI. *Exp. 13. Trypsin-free erepsin on glycyl-L-asparagine*

Digest: 0.5906 g. *glycyl-L-asparagine* neutralized and made up to 25 ml. with phosphate buffer of pH 7.8; 5 ml. of this solution removed for substrate control. Residual solution treated with 5 ml. of erepsin. The control showed no ammonia.

Time	Ammonia in 1 ml. of digestion as ml. <i>N</i> /280 acid	% amide split	ml. <i>N</i> /10 KOH for 2 ml. of digest	Increase in titre	% peptide split
0	0.18	0	3.68	0	0
4 hr.	0.22	0.14	4.38	0.7	35.2
13 hr.	0.64	1.64	4.99	1.31	65.6
18½ hr.	0.64	1.64	5.18	1.50	75.2
24 hr.	0.78	2.14	5.28	1.60	80.0
48 hr.	0.90	2.57	5.38	1.70	84.6
96 hr.	0.90	2.57	5.60	1.92	95.9
10 days	1.05	3.11	5.63	1.95	97.5
26 days	2.37	7.82	—	—	—

Exp. 14. Erepsin-free trypsin on glycyl-L-asparagine. Digest: 0.5906 g. of the substrate made up to 25 ml. with veronal buffer pH 8.4. 5 ml. removed for substrate control. Residual 20 ml. + 5 ml. trypsin formed the digest.

No peptide or amide hydrolysis at the end of 5 days.

Exp. 15. Pepsin on glycyl-L-asparagine. Digest: 0.5906 g. of *glycyl-L-asparagine* dissolved in 5 ml. *M*/5 HCl and made up to 25 ml. 5 ml. of this removed for substrate control and diluted to 6.5 ml. with *N*/20 HCl. Residual 20 ml. of the solution + 5 ml. pepsin formed the digest. No ammonia in enzyme controls.

No amide or peptide hydrolysis in 7 days.

Exp. 16. Papain-HCN on glycyl-L-asparagine. Digest: 0.463 g. substrate at pH 5 in citrate buffer containing 5 ml. papain-HCN solution in total volume 25 ml. No substrate control.

No amide or peptide hydrolysis in 9 days.

(5) *d- and l-Leucyl-l-asparagines*Table VII. *Exp. 17. Trypsin-free erepsin on d- and l-leucyl-l-asparagines*

Digest: 1.405 g. of the substrate dissolved in water and neutralized. 10 ml. of erepsin made up to 25 ml. with phosphate buffer pH 7.8. Controls showed no change and are not included in the table.

Time	Ammonia as ml. N/280 acid in 1 ml. digest	% amide split	ml. N/10 KOH for 2 ml. digest	Increase in titre value	% peptide split
0	0.5	0	3.61	—	—
6 hr.	—	0	4.09	0.48	12.0
12 hr.	0.56	0.10	4.16	0.55	13.75
24 hr.	0.54	0.10	4.21	0.60	15.0
36 hr.	0.70	0.36	4.23	0.62	15.5
54 hr.	0.88	0.68	4.27	0.66	16.5
5 days	1.21	1.26	4.28	0.67	16.75
10 days	1.70	2.14	—	—	—

Exp. 18. Erepsin-free trypsin on d- and l-leucyl-l-asparagines. Digest: 0.620 g. of the substrate dissolved in water neutralized and made up to 25 ml. with 10 ml. veronal buffer pH 8.4 and 5 ml. trypsin. No controls.

No amide or peptide hydrolysis in 6 days.

Exp. 19. Pepsin on d- and l-leucyl-l-asparagines. Digest: 0.878 g. of substrate dissolved in 25 ml. of water and sufficient N/20 HCl to bring to pH 1.8. 5 ml. removed for substrate control. Residual solution + 5 ml. pepsin formed the digest. Enzyme control showed no change.

No amide or peptide hydrolysis in 8 days.

Exp. 20. Papain-HCN on d- and l-leucyl-l-asparagines. Digest: 0.502 g. of the peptide dissolved in water and made up to 25 ml. with 5 ml. papain solution and 10 ml. citrate buffer pH 5. No substrate control.

No amide or peptide hydrolysis at the end of 7 days.

(6) *d-Leucyl-l-asparagine*

Exp. 21. Trypsin-free erepsin on d-leucyl-l-asparagine. Digest: 1.00 g. of substrate dissolved in water, neutralized, and made up to 25 ml. with sufficient phosphate buffer at pH 7.8. 5 ml. removed for substrate control. To the residual 20 ml., 5 ml. erepsin added.

No peptide hydrolysis in 12 days; amide split 0.21 %.

Exp. 22. Erepsin-free trypsin on d-leucyl-l-asparagine. Digest: 0.6342 g. of peptide dissolved in water, neutralized and made up to 25 ml. with 5 ml. enzyme and 10 ml. veronal buffer of pH 8.4.

In 7 days, amide split 0.25 %, peptide split nil.

(7) *Chloroacetyl-l-asparagine*Table VIII. *Exp. 23. Trypsin-free erepsin on chloroacetyl-l-asparagine*

Digest: 1.264 g. chloroacetyl-l-asparagine neutralized and made up to 25 ml. with phosphate buffer at pH 7.8. 10 ml. of this + 10 ml. erepsin diluted to 25 ml. formed the digest. Substrate and enzyme controls showed no change.

Time	Ammonia as ml. N/280 acid in 2 ml. digest	% amide split	ml. N/10 KOH for 2 ml. digest	% peptide split
0	0.09	—	1.43	—
6 hr.	0.15	0.22	1.65	11.3
18 hr.	0.18	0.33	2.01	29.9
24 hr.	0.24	0.55	2.04	31.5
48 hr.	0.73	2.69	2.07	33.0
96 hr.	1.40	5.15	—	—
6 days	—	—	2.92	76.8
10 days	2.76	10.20	—	—

Exp. 24. Erepsin-free trypsin on chloroacetyl-L-asparagine. Digest: 0.6515 g. substrate neutralized and made up to 25 ml. with veronal buffer pH 8.4. 5 ml. removed for substrate control. Residual 20 ml. + 5 ml. trypsin formed the digest.

In 7 days, amide split 0.2 %, peptide split nil.

(8) *Anhydroglycylasparagine*

Exp. 25. Trypsin-free erepsin on anhydroglycylasparagine. Digest: 0.7944 g. dissolved in 10 ml. phosphate buffer pH 7.8. 2 ml. removed for substrate control. Residue made up to 25 ml. with 10 ml. erepsin + water.

No amide or peptide split at the end of 6 days.

Exp. 26. Erepsin-free trypsin on anhydroglycylasparagine. Digest: 0.3086 g. substrate in 10 ml. water. 2 ml. removed for substrate control and the residue made up to 25 ml. with 5 ml. trypsin and 10 ml. buffer.

No amide or peptide split at the end of 7 days.

Exp. 27. Pepsin on anhydroglycylasparagine. Digest: the solution in Exp. 25 was used after inactivation of erepsin, adjustment of pH to 1.8 and addition of pepsin. The final strength of the solution was 0.041 N.

No amide or peptide split in 4 days.

Exp. 28. Papain-HCN on anhydroglycylasparagine. Digest: 0.2856 g. anhydroglycylasparagine dissolved in 10 ml. 2 ml. removed for substrate control and the residue diluted to 25 ml. after addition of 10 ml. citrate buffer pH 5 and 5 ml. papain solution.

No amide or peptide split at the end of 7 days.

SUMMARY

1. The amide group in leucinamide, asparagine and glycineamide is hydrolysed by trypsin-free erepsin, the rate of hydrolysis decreasing with the three substrates in the order given.

2. Trypsin-free erepsin also hydrolyses the peptide group in chloroacetyl-asparagine, but not the amide group in either this or other asparagine peptides examined.

3. Pepsin, erepsin-free trypsin and papain are without action upon the amide or peptide groups in glycineamide, leucinamide, asparagine, glycylasparagine or leucylasparagine.

4. Anhydroglycylasparagine (2:5-diketopiperazine acetamide) is not affected by any of the animal proteases or by papain.

5. The bearing of these results on (i) the specificity of the proteolytic enzymes, and (ii) the "amide" nitrogen of proteins is briefly discussed.

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