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Purines. XIV.[1] Selective Removal of N-Terminal Amino Acids from Peptides by the Use of Purin-6-yl Group at the N-Terminus[2]

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Introduction of the purin-6-yl group into oligopeptides IIa—f on the N-terminal nitrogen was effected by treating them with 6-chloropurine in boiling aq. 1-butanol containing triethylamine or in H_2O containing one eq. mole of NaOH at 90°. The yields of the purinylated peptides (IIIa—f) were 8—73%. Similar condensations of 4-chloro-6-methylpyrimidine with IIa and with glycine provided XII and XIII. In alternative synthesis of IIIa,d,e, (purin-6-yl)amino acids IVa and IVb were coupled with ethyl glycinate or glycylglycinate by the dicyclohexylcarbodiimide method, followed by hydrolysis of the resulting esters VIIIa,b,c. Condensation of α-naphthylamine with ethyl N-chloroacetylglycinate and alkaline hydrolysis of the product furnished N-(α-naphthyl)-glycylglycine (X).

Next the hydrolyses of dipeptides IIa,b,c, IIIa,b,c, X, and XII in boiling H_2O at various pH's were examined. At pH 7 all dipeptides were practically stable for at least 10 hr, but at pH 13 they were hydrolyzed at various rates. At pH 1 N-substituted dipeptides IIIa,b,c, X, and XII suffered hydrolysis more rapidly than did the parent dipeptides (IIa,b,c). Among the glycylglycine derivatives, purinyl derivative IIIa was hydrolyzed most rapidly. At pH 4 purinyl derivatives IIIa,b,c only underwent hydrolysis, whereas the other dipeptides remained unchanged even after 10 hr's reflux.

In the hydrolyses of the N-purinylated tri- (IIId,e) and tetrapeptides (IIIf) in H_2O at pH 4 and 100° for 5 hr, the N-terminal residues were almost selectively disconnected to the extent of 21—78%. It is suggested that the observed assistance of the purinyl substituent in hydrolytic cleavage of the N-terminal peptide bond is intramolecular in nature.

In connection with a need for an alternative to currently available methods[4] for the stepwise degradation of peptides, the N-terminal sequence determination utilizing a reagent which carries accessory functional groups has become of interest in recent years.[5] The catalyt-

1) Part XIII: T. Fujii and T. Saito, *Chem. Pharm. Bull.* (Tokyo), **21**, 1954 (1973).
2) A part of this work was presented before the 33rd Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, November 13, 1971.
3) Location: *13-1 Takara-machi, Kanazawa 920, Japan.*
4) J.L. Bailey, "Techniques in Protein Chemistry," 2nd ed., Elsevier Publishing Co., New York, N.Y., 1967, pp. 163—249.
5) *a*) R.A. Laursen, *J. Am. Chem. Soc.*, **88**, 5344 (1966); *b*) P. Edman and G. Begg, *European J. Biochem.*, **1**, 80 (1967); *c*) K.L. Kirk and L.A. Cohen, *J. Org. Chem.*, **34**, 384 (1969); *d*) *Idem, ibid.*, **34**, 390 (1969), and references cited; *e*) *Idem, ibid.*, **34**, 395 (1969); *f*) R.M. Lequin and H.D. Niall, *Biochim. Biophys. Acta*, **257**, 76 (1972).

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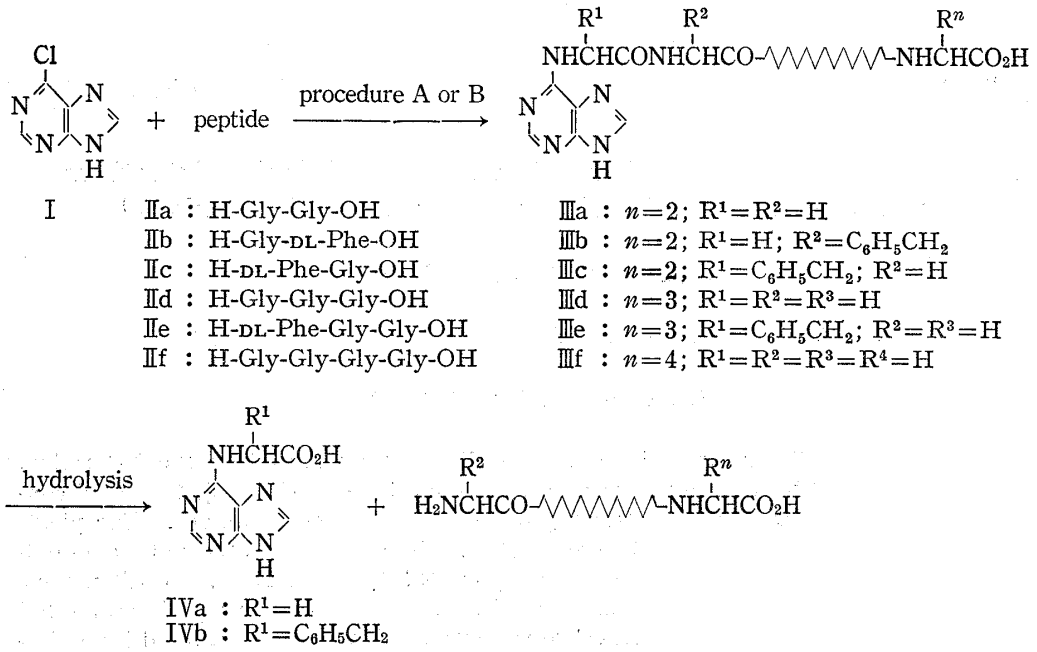
ic property of purines observed by Bruice and Schmir[6] in the hydrolysis of *p*-nitrophenyl acetate and facilitation of the hydrolysis of several dipeptides, reported by Kirk and Cohen,[5d] by intramolecular participation of the 5,7-dinitrobenzimidazol-4-yl group suggest the obvious analogy of hydrolytic, selective removal of N-terminal residues from peptides by nitrogen participation in the form of the purine ring. In this paper we wish to describe a method for introducing the purin-6-yl group into oligopeptides (type II) on the N-terminal amino group and hydrolysis study of the resulting purinylated peptides (type III) at various pH values.



I

IIa : H-Gly-Gly-OH
IIb : H-Gly-DL-Phe-OH
IIc : H-DL-Phe-Gly-OH
IId : H-Gly-Gly-Gly-OH
IIe : H-DL-Phe-Gly-Gly-OH
IIf : H-Gly-Gly-Gly-Gly-OH

IIIa : $n=2$; $R^1=R^2=H$
IIIb : $n=2$; $R^1=H$; $R^2=C_6H_5CH_2$
IIIc : $n=2$; $R^1=C_6H_5CH_2$; $R^2=H$
IIId : $n=3$; $R^1=R^2=R^3=H$
IIIe : $n=3$; $R^1=C_6H_5CH_2$; $R^2=R^3=H$
IIIf : $n=4$; $R^1=R^2=R^3=R^4=H$

IVa : $R^1=H$
IVb : $R^1=C_6H_5CH_2$

Chart 1

Our first objective was N-(purin-6-yl)glycylglycine (IIIa) which we hoped to prepare by condensation of glycylglycine (IIa) with 6-chloropurine (I)[7] utilizing a procedure patterned after that[8,9] employed for the synthesis of N^6-substituted adenines from I and amines or amino acids. Treatment of IIa with a slight excess of I in boiling 75% aqueous 1-butanol solution containing triethylamine for 12 hr (procedure A) furnished, after acidification (pH *ca.* 3), the desired product (IIIa) in 73% yield. This substance was characterized by correct analysis for $C_9H_{10}O_3N_6$, mass spectrum [*m/e* 250 (M^+)], infrared (IR) spectrum [ν_{max}^{KBr} cm^{-1}: 3435, 3275 (NH), 1650 (very broad, amide CO and COO^-)], and nuclear magnetic resonance (NMR) spectrum. The NMR spectrum in deuterated dimethyl sulfoxide exhibited two two-proton doublets ($J=6$ cps each) at 6.20 ($NHC\underline{H}_2CO_2H$) and 5.80 τ ($NHC\underline{H}_2CONH$), a dull one-proton triplet ($J=6$ cps) at 2.38 τ (purinyl-$N\underline{H}CH_2CONH$), two one-proton singlets at 1.86 and 1.92 τ (purine protons), and a broad one-proton peak at 1.7 τ ($CON\underline{H}CH_2$). However, the determination of the carboxylic (or zwitterionic) and $N_{(9)}$-H proton frequencies was hampered by the broadness of the signals. The most conclusive evidence for the presence

6) *a*) T.C. Bruice and G.L. Schmir, *J. Am. Chem. Soc.*, **80**, 148 (1958); *b*) T.C. Bruice and S.J. Benkovic, "Bioorganic Mechanisms," Vol. 1, W.A. Benjamin, Inc., New York, N.Y., 1966, p. 54.
7) *a*) A. Bendich, P.J. Russell, Jr., and J.J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954); *b*) A.G. Beaman and R.K. Robins, *J. Appl. Chem.*, **12**, 432 (1962); *c*) Y. Fujimoto and M. Naruse, Japan. Patent 6927649 (1969) [*Chem. Abstr.*, **68**, 49655*k* (1968)].
8) *a*) J. Žemlička and F. Šorm, *Collection Czech. Chem. Commun.*, **30**, 1880 (1965); *b*) S.M. Hecht, J.P. Helgeson, and T. Fujii, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, ed. by W.W. Zorbach and R.S. Tipson, Interscience Publishers, Inc., New York, N.Y., 1968, pp. 8—10.
9) *a*) C.E. Carter, *J. Biol. Chem.*, **223**, 139 (1956); *b*) H. Lettré and H. Ballweg, *Ann. Chem.*, **633**, 171 (1960); *c*) A. Ballio and V. Di Vittorio, *Gazz. Chim. Ital.*, **90**, 501 (1960) [*C. A.*, **55**, 15499*c* (1961)]; *d*) N. Ward, J. Wade, E.F. Walborg, Jr., and T.S. Osdene, *J. Org. Chem.*, **26**, 5000 (1961).

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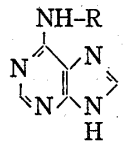
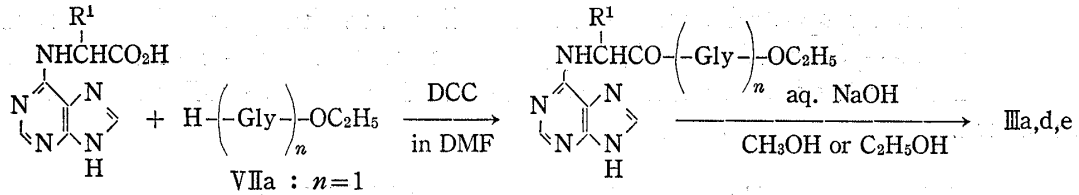
of the N^6-monosubstituted adenine structure in IIIa was provided by its ultraviolet (UV) spectra at various pH's, which resembled those of N-propyladenine (VI)[10] prepared by catalytic reduction of N-allyladenine (V)[10b] using hydrogen and Adams catalyst. Final identification as IIIa rested on its alternative synthesis as shown in Chart 3. The reaction sequence consisted of the coupling of N-(purin-6-yl)glycine (IVa)[9d] with glycine ethyl ester (VIIa) by the N,N'-dicyclohexylcarbodiimide (DCC) method in N,N-dimethylformamide solution (60% yield) and conversion of the resulting dipeptide ethyl ester (VIIIa) into IIIa on treating with methanolic sodium hydroxide at room temperature.



V: $R=CH_2CH=CH_2$
VI: $R=CH_2CH_2CH_3$

Chart 2



IVa : $R^1=H$
IVb : $R^1=C_6H_5CH_2$

VIIa : $n=1$
VIIb : $n=2$

VIIIa : $R^1=H$; $n=1$
VIIIb : $R^1=H$; $n=2$
VIIIc : $R^1=C_6H_5CH_2$; $n=2$

Chart 3

Condensation of glycyl-DL-phenylalanine (IIb) with I was accomplished under the same conditions as described above for IIa (procedure A), and N-(purin-6-yl)glycyl-DL-phenylalanine (IIIb) was obtained in 59% yield. In the case of DL-phenylalanylglycine (IIc), application of procedure A to the condensation with I was found to be incapable of effecting the reaction. However, this difficulty was overcome by heating a solution of the dipeptide (IIc) and I in water containing one equivalent mole of sodium hydroxide for 9—12 hr at 90° (procedure B), and it was possible to isolate N-(purin-6-yl)-DL-phenylalanylglycine (IIIc) in 30% yield. The structures IIIb and IIIc were also confirmed by analyses and by spectral properties as in the case of IIIa.

We next extended this purinylation reaction to include tri- and tetrapeptides. Reaction of glycylglycylglycine (IId) with 1.1 molar equivalents of I under the procedure A conditions led to N-(purin-6-yl)glycylglycylglycine (IIId) in 36% yield. In alternative synthesis of IIId, N-purinylated amino acid IVa was condensed with ethyl glycylglycinate (VIIb) by the DCC method to give the corresponding tripeptide ester (VIIIb). Hydrolysis of VIIIb leading to IIId was effected with sodium hydroxide in aqueous methanol at room temperature. However, the overall yield of IIId in this reaction sequence was poor. N-(Purin-6-yl)-DL-phenylalanylglycylglycine (IIIe) was prepared by the condensation of DL-phenylalanylglycylglycine (IIe) with I under the procedure B conditions or by the coupling of N-(purin-6-yl)-DL-phenylalanine (IVb)[9d] with VIIb by use of the DCC method, followed by alkaline hydrolysis of the resulting tripeptide ester (VIIIc). In either synthesis, however, the yield of IIIe was less satisfactory. A model compound chosen for tetrapeptides was glycylglycylglycylglycine (IIf), and this peptide reacted with I under the procedure A conditions to afford N-(purin-6-yl)-glycylglycylglycylglycine (IIIf) in 29% yield.

For a comparative study of hydrolysis of dipeptides, we tried to synthesize additional glycylglycine derivatives possessing aromatic groups of another type, for example, substituents roughly isosteric with or part-structurally similar to the purin-6-yl group, on the N-terminal

10) *a*) C.G. Skinner, W. Shive, R.G. Ham, D.C. Fitzgerald, Jr., and R.E. Eakin, *J. Am. Chem. Soc.*, **78**, 5097 (1956); *b*) F.S. Okumura, N. Enishi, H. Itoh, M. Masumura, and S. Kuraishi, *Bull. Chem. Soc. Japan*, **32**, 886 (1959).

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nitrogen. Thus, heating a mixture of α-naphthylamine (IX) and ethyl N-chloroacetylglycinate at 90° and alkaline hydrolysis of the resulting condensation product at room temperature produced N-(α-naphthyl)glycylglycine (X) in 48% yield. The IR spectrum of X in the solid state displayed a strong carboxyl band at 1724 cm^{-1}, suggesting the absence of the zwitterion form. This presents a striking contrast to the cases of the purinylated peptides (III) where no carboxyl CO band but a carboxylate band was observed. It is reasonable to assume that the predominance of the neutral form in X over the dipolar form is probably owing to the decrease in basicity of the N-terminal nitrogen caused by the attaching α-naphthyl group. The pyrimidyl-substituted glycylglycine (XII) was synthesized in 56% yield by the reaction of 4-chloro-6-methylpyrimidine (XI)[11] with IIa in 75% aqueous 1-butanol at reflux in the presence of triethylamine. A similar treatment of XI with glycine gave N-(6-methyl-4-pyrimidyl)glycine (XIII) in 77% yield. Proof of the correctness of structures X, XII, and XIII was furnished by spectral data described in Experimental part. In an attempt to prepare N-(imidazol-4-ylmethyl)glycylglycine (XV), condensation of 4-chloromethylimidazole (XIV)[12] with IIa was tried under various conditions. However, we failed in obtaining the desired product.

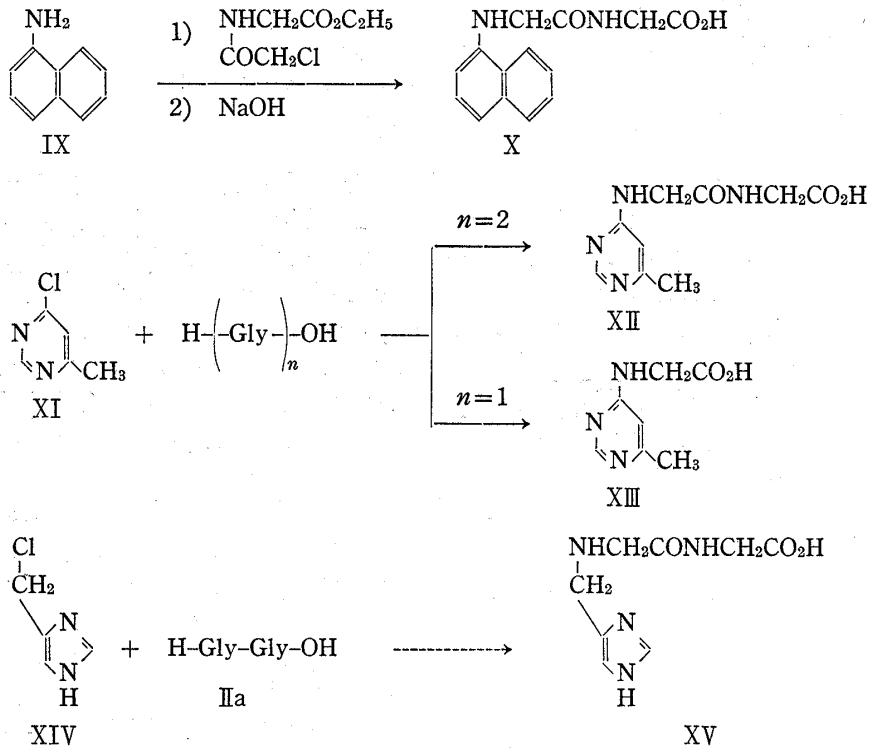

Chart 4

Next our attention was focused on hydrolysis study of the peptide derivatives (III, X, and XII) thus prepared and of the parent peptides (II) unsubstituted on the N-terminal amino group. We first followed the progress of the hydrolysis of dipeptide IIIa in boiling water at different pH's by measuring the amount of the C-terminal glycine that split off (Chart 1). At pH 1 the hydrolysis proceeded rapidly and was complete within 5 hr, whereas at pH 7 the starting peptide (IIIa) almost entirely remained unchanged even after 10 hr's reflux. The rates of the reaction at pH 4 and 13 were found to be moderate. For comparison,

11) J.R. Marshall and J. Walker, *J. Chem. Soc.*, **1951**, 1004.
12) *a*) R.A. Turner, C.F. Huebner, and C.R. Scholz, *J. Am. Chem. Soc.*, **71**, 2801 (1949), and references cited; *b*) J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 3, John Wiley & Sons, Inc., New York, N.Y., 1961, pp. 1986—1987.

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the hydrolyses of the other dipeptides (IIa,b,c, IIIb,c, X, and XII) were also studied separately under the identical reaction conditions, and the extent of the hydrolysis at an early stage in each reaction was adopted as a quick guide to the reaction rate. It may be seen from Table I that at pH 1 the N-substituted dipeptides (IIIa,b,c, X, and XII) hydrolyzed more rapidly than did the corresponding parent dipeptides (IIa,b,c). Comparison of the initial rates for the glycylglycine derivatives suggests the sequence IIa<XII<X<IIIa for increasing reactivity of the individual compounds. This implies that the purinyl group has the most profound effect among the substituents tested. At pH 4 both the α-naphthyl and 6-methyl-4-pyrimidyl substituents appeared to have no or little accelerating effect on the hydrolytic cleavage of the N-substituted glycylglycines (X and XII) to which they are attached, whereas the purin-6-yl substituent in IIIa still exercised a marked effect. This suggests that the imidazole moiety in the purin-6-yl group plays an important role in the hydrolysis. Since IIIc seems to undergo hydrolysis faster than its isomer (IIIb) (Table I), the ease with which the peptide bond of the N-purinylated dipeptides is cleaved may be further influenced by the sequence of amino acids and especially by the nature of the N-terminal residue. At pH 7 all the peptides tested were practically stable for at least 10 hr, and at pH 13 they underwent hydrolysis at various rates, so that the specificity of the purin-6-yl group in the hydrolysis as found in the acid region could not be recognized.

Table I. Hydrolysis of 1 mM Dipeptides in Boiling Water

Dipeptide	Reaction time (hr)	Hydrolysis (%) pH value[a]			
		1	4	7	13
(Purin-6-yl)-Gly-Gly-OH (IIIa)	1	72	13	0	40
	5	101	42	0	82
	10	104	56	0	85
(α-Naphthyl)-Gly-Gly-OH (X)	1	38	0	0	34
	5	76	0	0	74
	10	78	0	0	84
(6-Methyl-4-pyrimidyl)-Gly-Gly-OH (XII)	1	9	0	0	56
	5	34	5	0	95
	10	52	8	0	—
H-Gly-Gly-OH (IIa)	10	12	0	0	92
(Purin-6-yl)-Gly-DL-Phe-OH (IIIb)	1	32	6	0	1
	5	81	17	0	5
	10	—	25	0	9
H-Gly-DL-Phe-OH (IIb)	10	5	0	0	33
(Purin-6-yl)-DL-Phe-Gly-OH (IIIc)	1	98	71	0	6
	5	103	90	4	42
	10	—	—	13	59
H-DL-Phe-Gly-OH (IIc)	10	14	0	0	39

a) Solutions used for the hydrolysis were 0.1N aq. HCl (pH 1), 0.08M CH_3CO_2H–0.02M CH_3CO_2Na buffer (pH 4), 0.01M KH_2PO_4–0.01M Na_2HPO_4–0.08M KCl buffer (pH 7), and 0.1N aq. NaOH (pH 13).

In order to learn whether the observed assistance of the purinyl substituent in hydrolytic cleavage of the dipeptide derivatives is intra- or intermolecular in nature, a mixture of equimolecular amounts of IIa and any of adenine, VI, or IVa was treated with boiling water under the same conditions as described above. In all cases the results obtained were just comparable to those described above for the hydrolysis of IIa alone. Consequently, it may be assumed that the acceleration of hydrolysis of the purinylated dipeptides (IIIa,b,c) is ascribed to intramolecular participation of the ring-nitrogen atom(s) of the substituent. However, lack of kinetic study at present discourages us to discuss a more detailed mechanism.

Probably the most striking aspect of these hydrolyses is the almost complete specificity observed at pH 4 for the purinylated derivatives (IIIa,b,c). As shown in Table I, the parent dipeptides (IIa,b,c) were resistant to hydrolysis at this pH value, in contrast to the suscep-

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tibility of the corresponding N-purinylated ones. Therefore, if these reaction conditions are applied to hydrolysis of a longer peptide carrying the purin-6-yl substituent on the N-terminal nitrogen, it should be expected that the N-terminal residue is selectively removed from the peptide. To substantiate this point, the purinylated oligopeptides (IIId,e,f) were separately heated in water at pH 4 and 100° for 5 hr. Chromatographic and electrophoretic analyses of the reaction mixtures disclosed the results recorded in Table II. In the case of tripeptide IIId, the N-terminal residue (IVa) was removed in 29% yield to give dipeptide IIa. Formation of a small amount (1.3% yield) of glycine and a trace of IIIa indicates that the cleavage at the C-terminal peptide bond also took place to a small extent. Although some of the N-(purin-6-yl)glycine (IVa) and of the glycine that formed must have been derived from the primary product IIIa, the extent should be not more than 1.3%. Accordingly, the specificity of the purinyl substituent for cleavage of the N-terminal peptide bond may be regarded as being considerably high. Similar results with tripeptide IIIe and with tetrapeptide IIIf have established the generality of this selective removal of the N-terminal residue. It is noted that the purinylated N-terminal phenylalanine moiety was disconnected from IIIe more rapidly than was detached the purinylated N-terminal glycine moiety from IIId. Such a tendency has also been observed for a set of dipeptides IIIc and IIIb (Table I).

Table II. Hydrolysis of 10 mM N-(Purin-6-yl)-oligopeptides (IIId,e,f) in Water at pH 4 and 100° for 5 hr

Compound[a]	Product[a]	Yield (%)
Pu-Gly-Gly-Gly-OH (IIId)	*Pu*-Gly-OH (IVa)	29
	H-Gly-Gly-OH (IIa)	—
	H-Gly-OH	1.3
	Pu-Gly-Gly-OH (IIIa)	trace
Pu-DL-Phe-Gly-Gly-OH (IIIe)	*Pu*-DL-Phe-OH (IVb)	78
	H-Gly-Gly-OH (IIa)	—
	H-Gly-OH	2.2
	Pu-DL-Phe-Gly-OH (IIIc)	trace
Pu-Gly-Gly-Gly-Gly-OH (IIIf)	*Pu*-Gly-OH (IVa)	21
	H-Gly-Gly-Gly-OH (IId)	—
	H-Gly-OH	3.9
	Pu-Gly-Gly-Gly-OH (IIId)	trace
	possibly other products	trace

a) The symbol *Pu* designates the purin-6-yl group.

In conclusion, the method described above for selective removal of N-terminal amino acids from oligopeptides by employing the purin-6-yl group at the N-terminus has demonstrated its potential utility in the stepwise degradation of polypeptides from the N-terminus. Further investigation along this line is now under way.

Experimental[13]

Preparation of Unsubstituted Peptides IIa—f——All unsubstituted peptides (IIa—f) used in this study were synthesized according to the adequate general procedures[15] and recrystallized from aq. ethanol. Their purities were checked by paper chromatography and elemental analyses.

13) All melting points are corrected. Paper chromatographies were developed as described previously,[14] and spots were detected by means of UV absorbance and/or the ninhydrin test. See also ref. 14 for details of instrumentation and measurement. The following abbreviations are used: b=broad, d=doublet, DMSO=dimethyl sulfoxide, m=multiplet, q=quartet, s=singlet, t=triplet, sh=shoulder. We are grateful to Mr. Y. Itatani and Misses M. Imai, S. Toyoshima, and T. Tsuji at Kanazawa University for microanalyses and NMR and mass spectral data.

14) T. Fujii, T. Itaya, and S. Moro, *Chem. Pharm. Bull.* (Tokyo), **20**, 958 (1972).

15) *a*) J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley & Sons, Inc., New York, N.Y., 1961, Chapter 10; *b*) T. Shiba, "Chemistry of Proteins," Vol. 1 (in Japanese), ed. by S. Akabori, T. Kaneko, and K. Narita, Kyoritsu Shuppan Co., Tokyo, 1969, Chapter 5.

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N-(Purin-6-yl)glycylglycine (IIIa)——i) Procedure A: A stirred mixture of IIa (3.21 g, 24.3 mmoles), I (4.11 g, 26.6 mmoles), triethylamine (10.9 g, 108 mmoles), 1-butanol (48 ml), and H_2O (16 ml) was heated at reflux for 12 hr. The reaction mixture was evaporated *in vacuo* to leave a syrup, which was dissolved in a little H_2O. The aq. solution was made acid (pH 3—4) with 50% aq. formic acid, and the precipitates that resulted were filtered off, washed with a little cold H_2O, and dried to give a monohydrate of IIIa (4.73 g, 73% based on the IIa used), shown to be homogeneous by paper chromatography. Recrystallization from 50% (v/v) aq. ethanol and drying over P_2O_5 at room temp. and 5 mm Hg for 72 hr furnished an analytical sample of IIIa·H_2O as almost colorless, minute crystals, mp 266° (decomp.). *Anal.* Calcd. for $C_9H_{10}O_3N_6 \cdot H_2O$: C, 40.30; H, 4.51; N, 31.33. Found: C, 40.22; H, 4.62; N, 31.24. Further drying of the monohydrate over P_2O_5 at 110° and 2 mm Hg for 16 hr produced an anhydrous sample, mp 270° (decomp.). *Anal.* Calcd. for $C_9H_{10}O_3N_6$: C, 43.20; H, 4.03; N, 33.59. Found: C, 43.47; H, 4.23; N, 33.62. UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 276 mμ (ε 17100); $\lambda_{max}^{H_2O}$ (pH 7)[17] 267 (16600); $\lambda_{max}^{H_2O}$ (pH 13)[18] 273 (15700), 280 (sh) (11700). For IR, NMR, and mass spectral data, see Theoretical part.

ii) Hydrolysis of Dipeptide Ester VIIIa: A mixture of VIIIa (101 mg, 0.363 mmole) and 0.01N methanolic NaOH (43 ml) was stirred at room temp. for 5 hr. Distillation of the solvent under vacuum left a syrup, which was dissolved in H_2O (5 ml). The aq. solution was adjusted to pH 3 with 50% aq. formic acid, and the precipitates that formed were filtered off and recrystallized from 50% (v/v) aq. ethanol to provide colorless micro-needles, which were dried in the same way as described above. The resulting anhydrous sample, mp 270° (decomp.), was identical (by mixed melting-point test, paper chromatography, and UV spectrum) with the one obtained by procedure A.

N-(Purin-6-yl)glycylglycine Ethyl Ester (VIIIa)——To a solution of N-(purin-6-yl)glycine hemihydrate[9d] (IVa·1/2 H_2O: 101 mg, 0.5 mmole) in N,N-dimethylformamide (DMF) (20 ml) was added dicyclohexylcarbodiimide (DCC) (103 mg, 0.5 mmole), and the mixture was stirred at room temp. for 30 min. Then a solution of VIIa·HCl (69.8 mg, 0.5 mmole) in DMF (10 ml) containing triethylamine (50 mg, 0.5 mmole) was added under cooling. The resulting mixture was kept stirring in an ice bath for 8 hr. The precipitates that formed were collected by filtration and washed with a little DMF. The filtrate and washings were combined and evaporated *in vacuo* to dryness, leaving a pale yellowish solid. The residue was chromatographed on a column packed with silica gel (16.5 g). Elution of the column with ethanol–ethyl acetate (1: 6, v/v) and concentration of the fractions containing VIIIa gave a colorless solid (83 mg, 60%). Recrystallization from 30% (v/v) aq. ethanol produced colorless needles, mp 238—239° (decomp.); UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 276 mμ (ε 17300); $\lambda_{max}^{H_2O}$ (pH 7)[17] 266 (17000); $\lambda_{max}^{H_2O}$ (pH 13)[18] 273 (16100), 280 (sh) (12000); IR ν_{max}^{Nujol} cm^{-1}: 3385, 3290 (NH), 1740 (ester CO), 1681 (amide CO); NMR (DMSO-d_6) τ: 8.79 (3H, t, J=7 cps, $CO_2CH_2C\underline{H}_3$), 6.12 (2H, d, J=6 cps, $NHC\underline{H}_2CO_2$), 5.90 (2H, q, J=7 cps, $CO_2C\underline{H}_2CH_3$), 5.76 (2H, d, J=6 cps, $NHC\underline{H}_2CONH$), 2.30 (1H, t, J=6 cps, purinyl–N$\underline{H}$$CH_2$), 1.85 and 1.78 (1H each, s, purine protons), 1.68 (1H, t, J=6 cps, $CONHCH_2$), −2.9 (1H, b, $N_{(9)}$–H); Mass Spectrum *m/e* 278 (M^+). *Anal.* Calcd. for $C_{11}H_{14}O_3N_6$: C, 47.48; H, 5.07; N, 30.20. Found: C, 47.39; H, 5.13; N, 30.07.

N-Propyladenine (VI)——A solution of N-allyladenine[10b] (V: 504 mg, 2.88 mmoles) in 80% (v/v) aq. ethanol (25 ml) was hydrogenated over Adams catalyst (50 mg) at room temp. and atmospheric pressure; the reaction was complete within 1 hr, absorbing one equivalent mole of H_2. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo* to dryness to leave a slightly yellowish solid. The residue was then purified by column chromatography [silica gel (20 g), chloroform–ethanol (6: 1, v/v)]. The fractions shown to be homogeneous by thin-layer chromatography (TLC) were combined and evaporated to give a colorless solid (399 mg, 78%), mp 244—245°. Recrystallization from H_2O provided an analytical sample of VI as colorless needles, mp 245—246° [lit. mp 240—241° (decomp.);[10a] mp 234°[10b]]; UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 272 mμ (ε 15300); $\lambda_{max}^{H_2O}$ (pH 7)[17] 270 (16400); $\lambda_{max}^{H_2O}$ (pH 13)[18] 275 (16500), 282 (sh) (12900); NMR (DMSO-d_6) τ: 9.08 (3H, t, J=7 cps, $CH_2C\underline{H}_3$), 8.37 (2H, m, $CH_2C\underline{H}_2CH_3$), 6.53 (2H, b, $NHC\underline{H}_2CH_2$), 2.39 (1H, b, t, J=6 cps, N$\underline{H}$$CH_2$), 1.87 and 1.78 (1H each, s, purine protons). *Anal.* Calcd. for $C_8H_{11}N_5$: C, 54.22; H, 6.26; N, 39.52. Found: C, 54.45; H, 6.22; N, 39.49.

N-(Purin-6-yl)glycyl-DL-phenylalanine (IIIb)——A mixture of IIb (4.00 g, 18 mmoles), I (3.06 g, 19.8 mmoles), triethylamine (10.0 g, 98.8 mmoles), 1-butanol (36 ml), and H_2O (12 ml) was heated under reflux with stirring for 13 hr. The resulting mixture was evaporated *in vacuo* to dryness, and the residue was dissolved in H_2O (*ca.* 30 ml). The pH of the aq. solution was adjusted to 3 with 10% aq. HCl. The tan precipitates that formed were filtered off, washed with a little H_2O, and dissolved in hot 50% (v/v) aq. ethanol. The hot solution was treated with charcoal and filtered. On cooling, the filtrate produced IIIb·H_2O (3.81 g, 59%) as pale yellowish, minute scales, mp 161° (decomp.), shown to be homogeneous on a TLC plate. Recrystallization from 50% (v/v) aq. ethanol and drying over P_2O_5 at 50° and 3 mm Hg for 17 hr afforded an analytical sample of the monohydrate, mp 162° (decomp.). *Anal.* Calcd. for $C_{16}H_{16}O_3N_6 \cdot H_2O$: C, 53.62; H, 5.06; N, 23.45. Found: C, 53.56; H, 5.13; N, 23.34. Further drying of the monohydrate over P_2O_5

16) Determined in 0.1N aq. HCl.
17) Determined in 0.005M phosphate buffer.
18) Measured in 0.1N aq. NaOH.

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at 110° and 2 mm Hg for 16 hr gave an anhydrous sample, mp 162° (decomp.); UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 278 mμ (ε 15400); $\lambda_{max}^{H_2O}$ (pH 7)[17] 267 (14500); $\lambda_{max}^{H_2O}$ (pH 13)[18] 274 (13600), 281 (sh) (10200); IR ν_{max}^{KBr} cm^{-1}: 3350, 3270 (NH), 1650 (very b, amide CO and COO$^-$); NMR (DMSO-d_6) τ: 6.94 (2H, m, $C_6H_5C\underline{H}_2CH$), 5.84 (2H, d, J=6 cps, $NHC\underline{H}_2CO$), 5.42 [1H, m, $NHC\underline{H}(CH_2C_6H_5)CO_2H$], 2.83 (5H, s, phenyl protons), 2.36 (1H, b, t, J=6 cps, purinyl-$N\underline{H}CH_2$), 1.86 and 1.80 (1H each, s, purine protons), 2.0—1.7 (1H, b, $CON\underline{H}CH$); Mass Spectrum *m/e* 340 (M^+). *Anal.* Calcd. for $C_{16}H_{16}O_3N_6$: C, 56.46; H, 4.74; N, 24.70. Found: C, 56.26; H, 4.90; N, 24.81.

N-(Purin-6-yl)-DL-phenylalanylglycine (IIIc)——A stirred mixture of IIc (3.11 g, 14 mmoles) and I (2.38 g, 15.4 mmoles) in H_2O (40 ml) containing 1N aq. NaOH (14 ml) was kept at 90° for 9 hr. After having been cooled, the reaction mixture was adjusted to pH 3 with 10% aq. HCl. The crude IIIc that precipitated was treated in the same way as described above for IIIb, and a hemihydrate of IIIc (1.45 g, 30%) was obtained as slightly yellowish needles, mp 189° (decomp.). For analysis, this sample was recrystallized from 50% (v/v) aq. ethanol and the resulting colorless minute needles, mp 194° (decomp.), were dried over P_2O_5 at 50° and 2 mm Hg for 5 hr. *Anal.* Calcd. for $C_{16}H_{16}O_3N_6 \cdot 1/2H_2O$: C, 55.00; H, 4.91; N, 24.06. Found: C, 55.33; H, 5.04; N, 23.92. UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 279 mμ; $\lambda_{max}^{H_2O}$ (pH 7)[17] 268; $\lambda_{max}^{H_2O}$ (pH 13)[18] 274, 280 (sh); IR ν_{max}^{KBr} cm^{-1}: 3330, 3290 (NH), 1650 (amide CO), 1615 (COO$^-$); NMR (DMSO-d_6) τ: 6.83 (2H, b, m, $C_6H_5C\underline{H}_2CH$), 6.19 (2H, d, J=6 cps, $NHC\underline{H}_2CO_2H$), 4.80 [1H, b, m, $NHC\underline{H}(CH_2C_6H_5)CO$], 3.0—2.3 [6H, m, phenyl protons and $N\underline{H}CH(CH_2C_6H_5)CO$], 1.84 and 1.80 (1H each, s, purine protons), 1.48 (1H, b, t, J=6 cps, $CON\underline{H}CH_2$); Mass Spectrum *m/e* 340 (M^+).

N-(Purin-6-yl)glycylglycylglycine (IIId)——i) Procedure A: A mixture of IId (2.94 g, 15.5 mmoles), I (2.64 g, 17.1 mmoles), triethylamine (7.83 g, 77.4 mmoles), 1-butanol (30 ml), and H_2O (10 ml) was allowed to react in the manner as described above for IIIa. Work-up of the reaction mixture was also carried out similarly. Recrystallization of the resulting crude IIId from 50% (v/v) aq. ethanol provided colorless minute needles (1.69 g, 36%), mp 257° (decomp.), which were dried over P_2O_5 at 105° and 1 mm Hg for 16 hr for analysis. *Anal.* Calcd. for $C_{11}H_{13}O_4N_7$: C, 42.99; H, 4.26; N, 31.91. Found: C, 42.78; H, 4.36; N, 31.81. UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 276 mμ (ε 17200); $\lambda_{max}^{H_2O}$ (pH 7)[17] 267 (16600); $\lambda_{max}^{H_2O}$ (pH 13)[18] 273 (15600), 279 (sh) (12100); IR ν_{max}^{Nujol} cm^{-1}: 3380, 3280 (NH), 1650 (very b, amide CO and COO$^-$); NMR (DMSO-d_6) τ: 6.28 (4H, overlapped d, J=6 cps, $NHC\underline{H}_2CONHC\underline{H}_2CO_2H$), 5.87 (2H, d, J=6 cps, purinyl-$NHC\underline{H}_2CO$), 2.32 (1H, b, t, J=6 cps, purinyl-$N\underline{H}CH_2CO$), 1.90 and 1.84 (1H each, s, purine protons), 2.1—1.55 (2H, b, $CON\underline{H}CH_2CON\underline{H}CH_2CO_2H$).

ii) Hydrolysis of Tripeptide Ester VIIIb: A solution of VIIIb (95 mg, 0.28 mmole) in a mixture of 1N aq. NaOH (0.3 ml), H_2O (0.7 ml), and methanol (2 ml) was kept at room temp. for 5 hr. The pH of the solution was adjusted to 3 with 10% aq. HCl, and the precipitates that resulted were filtered off, washed with a little H_2O, and recrystallized from 50% (v/v) aq. ethanol to give slightly yellowish, minute needles, mp 257° (decomp.), after dried over P_2O_5. Identity of this sample with the one obtained by procedure A was established by mixed melting-point test, paper chromatography, TLC, and UV and NMR spectra.

N-(Purin-6-yl)glycylglycylglycine Ethyl Ester (VIIIb)——To a solution of IVa·1/2H_2O[9d] (809 mg, 4 mmoles) in DMF (25 ml) was added DCC (825 mg, 4 mmoles), and the mixture was stirred in an ice bath for 30 min. Then VIIb·HCl (784 mg, 4 mmoles) and triethylamine (405 mg, 4 mmoles) were successively added. The resulting mixture was kept stirring under cooling for 5 hr and then at room temp. overnight. The insoluble solid that separated out was removed by filtration and the filtrate was evaporated *in vacuo* to dryness. The resulting residue was washed with ether and dissolved in a little 10% aq. HCl. The aq. solution was filtered in order to remove insoluble material. The filtrate was adjusted to pH 7—8 with 10% aq. Na_2CO_3, and the precipitates that resulted were filtered off and recrystallized from aq. ethanol to give VIIIb as slightly yellowish needles (154 mg, 12%), mp 237° (decomp.); UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 276 mμ (ε 16400); $\lambda_{max}^{H_2O}$ (pH 7)[17] 266 (15900); $\lambda_{max}^{H_2O}$ (pH 13)[18] 273 (14700), 280 (sh) (11100); IR ν_{max}^{Nujol} cm^{-1}: 3295, 3195 (NH), 1748 (ester CO), 1640 (b, amide CO); NMR (DMSO-d_6) τ: 8.82 (3H, t, J=7 cps, $CO_2CH_2C\underline{H}_3$), 6.23 (4H, overlapped d, J=6 cps, $NHC\underline{H}_2CONHC\underline{H}_2CO_2$), 5.92 (2H, q, J=7 cps, $CO_2C\underline{H}_2CH_3$), 5.83 (2H, d, J=6 cps, purinyl-$NHC\underline{H}_2CO$), 2.35 (1H, b, t, J=6 cps, purinyl-$N\underline{H}CH_2$), 1.88 and 1.82 (1H each, s, purine protons), 2.0—1.5 (2H, b, $CON\underline{H}CH_2CON\underline{H}CH_2$); Mass Spectrum *m/e* 335 (M^+). *Anal.* Calcd. for $C_{13}H_{17}O_4N_7$: C, 46.56; H, 5.11; N, 29.24. Found: C, 46.58; H, 5.30; N, 29.12.

N-(Purin-6-yl)-DL-phenylalanylglycylglycine (IIIe)——i) Procedure B: A mixture of IIe (2.79 g, 10 mmoles), I (1.70 g, 11 mmoles), 1N aq. NaOH (10 ml), and H_2O (40 ml) was stirred at 90° for 12 hr. Work-up of the reaction mixture and recrystallization of the crude product were carried out as described above for IIIc. A pure sample of IIIe was obtained in 8% yield as almost colorless micro-needles, mp 240° (decomp.); UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 280 mμ; $\lambda_{max}^{H_2O}$ (pH 7)[17] 268; $\lambda_{max}^{H_2O}$ (pH 13)[18] 274, 280 (sh); IR ν_{max}^{Nujol} cm^{-1}: 3255, 3135 (NH), 1700—1620 (very b, amide CO and COO$^-$); NMR (DMSO-d_6) τ: 6.8 (2H, b, m, $C_6H_5C\underline{H}_2CH$), 6.21 (4H, overlapped d, J=6 cps, $NHC\underline{H}_2CONHC\underline{H}_2CO_2H$), 4.8 [1H, b, $NHC\underline{H}(CH_2C_6H_5)CO$], 2.80 (5H, m, phenyl protons), 2.57 (1H, purinyl-$N\underline{H}CH_2$), 1.95 (1H, b, t, J=6 cps, $CON\underline{H}CH_2CO$), 1.90 and 1.86 (1H each, s, purine protons), 1.56 (1H, b, t, $CON\underline{H}CH_2CO_2H$). *Anal.* Calcd. for $C_{18}H_{19}O_4N_7$: C, 54.40; H, 4.82; N, 24.68. Found: C, 54.63; H, 4.97; N, 24.47.

ii) Hydrolysis of Tripeptide Ester VIIIc: A solution of VIIIc (242 mg, 0.57 mmole) in a mixture of ethanol (50 ml) and 1N aq. NaOH (0.8 ml) was kept at room temp. for 4 hr and then evaporated *in vacuo*

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to dryness. The residue was dissolved in a little H_2O and the aq. solution was adjusted to pH 3—4 with 10% aq. HCl. The precipitates that formed were filtered off and recrystallized from 50% (v/v) aq. ethanol to give colorless minute needles (65 mg, 29%), mp 239° (decomp.), identical (by mixed melting-point test, paper chromatography, and UV, IR, and NMR spectra) to the sample of IIIe prepared by means of procedure B.

N-(Purin-6-yl)-DL-phenylalanylglycylglycine Ethyl Ester (VIIIc)——Coupling of IVb[9d] with VIIb·HCl by the DCC method and purification of the product were carried out in the manner as described above for VIIIa. A pure sample of VIIIc was obtained in 36% yield as colorless needles, mp 137—139°; UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 280 mμ (ε 17800); $\lambda_{max}^{H_2O}$ (pH 7)[17] 268 (16400); $\lambda_{max}^{H_2O}$ (pH 13)[18] 275 (14700), 280 (sh) (12100); IR ν_{max}^{Nujol} cm^{-1}: 3285 (b, NH), 1730 (ester CO), 1660 (amide CO); NMR (DMSO-d_6) τ: 8.79 (3H, t, J=7 cps, $CO_2CH_2C\underline{H}_3$), 6.8 (2H, b, m, $C_6H_5C\underline{H}_2CH$), 6.3—6.0 (4H, m, $NHC\underline{H}_2CONHC\underline{H}_2CO_2$), 5.86 (2H, q, J=7 cps, $CO_2C\underline{H}_2CH_3$), 4.8 [1H, b, $NHC\underline{H}(CH_2C_6H_5)CO$], 2.70 (5H, m, phenyl protons), 2.47 [1H, b, purinyl-N$\underline{H}$CH($CH_2C_6H_5$)CO], 1.80 and 1.76 (1H each, s, purine protons), 1.7 (1H, b, CON$\underline{H}$$CH_2$CONH), 1.49 (1H, b, t, CON$\underline{H}$$CH_2CO_2$); Mass Spectrum m/e 425 (M^+). *Anal.* Calcd. for $C_{20}H_{23}O_4N_7$: C, 56.46; H, 5.45; N, 23.05. Found: C, 56.17; H, 5.57; N, 22.85.

N-(Purin-6-yl)glycylglycylglycylglycine (IIIf)——A mixture of IIf (539 mg, 2.19 mmoles), I (339 mg, 2.19 mmoles), triethylamine (2.22 g, 21.9 mmoles), 1-butanol (9 ml), and H_2O (3 ml) was heated at reflux for 12 hr. Work-up of the reaction mixture in a manner similar to that described for IIIa (procedure A) afforded IIIf (229 mg, 29%) as slightly yellowish, minute crystals, mp 238° (decomp.); UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 276 mμ; $\lambda_{max}^{H_2O}$ (pH 7)[17] 266; $\lambda_{max}^{H_2O}$ (pH 13)[18] 273, 279 (sh); IR ν_{max}^{Nujol} cm^{-1}: 3305 (NH), 1670 (sh), 1637 (b) (amide CO and COO^-); NMR (DMSO-d_6) τ: 6.3 (6H, b, $CONHC\underline{H}_2CONHC\underline{H}_2CONHC\underline{H}_2CO_2H$), 5.86 (2H, dull d, purinyl-$NHC\underline{H}_2CO$), 2.36 (1H, dull t, purinyl-N$\underline{H}$$CH_2$), 2.2—1.5 (3H, three NH's), 1.95 and 1.88 (1H each, s, purine protons).

N-(α-Naphthyl)glycylglycine (X)——An intimate mixture of IX (5.27 g, 36.8 mmoles) and ethyl N-chloroacetylglycinate[19] (3.00 g, 16.7 mmoles) was heated with stirring in an oil bath kept at 90° for a few min. After cooling, ether (50 ml) and 1N aq. NaOH (40 ml) were added and the mixture was stirred at room temp. for 2 hr. The aq. layer was separated, washed with ether, and made acid (pH 3) with 10% aq. HCl. The crystals that precipitated were filtered off, washed with H_2O, and dried. This sample (2.07 g, 48% based on the ester used), mp 166—167° (decomp.) was found to be homogeneous by paper chromatography and TLC. Recrystallization from 30% (v/v) aq. ethanol using charcoal produced an analytical sample of X as slightly violet plates, mp 169—170° (decomp.); UV $\lambda_{max}^{C_2H_5OH}$ mμ (ε): 246 (19600), 329 (7000); IR ν_{max}^{Nujol} cm^{-1}: 3430, 3380 (NH), 1724 (COOH), 1621 (amide CO); Mass Spectrum m/e 258 (M^+). *Anal.* Calcd. for $C_{14}H_{14}O_3N_2$: C, 65.10; H, 5.46; N, 10.85. Found: C, 64.96; H, 5.40; N, 10.77.

N-(6-Methyl-4-pyrimidyl)glycylglycine (XII)——A mixture of IIa (3.96 g, 30 mmoles), XI[11] (4.25 g, 33.1 mmoles), triethylamine (15.2 g, 150 mmoles), 1-butanol (45 ml), and H_2O (15 ml) was refluxed for 9.5 hr. The resulting solution was evaporated *in vacuo* to dryness to leave a yellowish residue, which was dissolved in H_2O. The aq. solution was washed with ether and evaporated again *in vacuo* to dryness. Recrystallization of the resulting residue from 50% (v/v) aq. ethanol using charcoal afforded XII (3.75 g, 56%) as colorless needles, mp 262° (decomp.); UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 259 mμ (ε 18000); $\lambda_{max}^{H_2O}$ (pH 7)[17] 242 (13700), 263 (sh) (5800); $\lambda_{max}^{H_2O}$ (pH 12)[20] 241 (14400), 265 (sh) (4300); IR ν_{max}^{Nujol} cm^{-1}: 3280, 3160 (NH), 1650 (b, amide CO and COO^-); NMR (DMSO-d_6) τ: 7.75 (3H, s, CH_3), 6.22 (2H, d, J=6 cps, $NHC\underline{H}_2CO_2H$), 6.03 (2H, d, J=6 cps, $NHC\underline{H}_2$-CONH), 3.55 (1H, b, $H_{(5)}$), 2.47 (1H, b, t, J=6 cps, pyrimidyl-N$\underline{H}$$CH_2CO$), 1.75 (2H, b, $H_{(2)}$ and CON$\underline{H}$$CH_2$); Mass Spectrum m/e 224 (M^+). *Anal.* Calcd. for $C_9H_{12}O_3N_4$: C, 48.21; H, 5.39; N, 24.99. Found: C, 48.04; H, 5.44; N, 24.93.

N-(6-Methyl-4-pyrimidyl)glycine (XIII)——A mixture of glycine (3.00 g, 40 mmoles), XI[11] (5.66 g, 44 mmoles), triethylamine (20.2 g, 200 mmoles), 1-butanol (60 ml), and H_2O (20 ml) was heated at reflux for 11 hr. The reaction mixture was worked up as described above for XII, affording XIII (5.13 g, 77%) as colorless needles, mp 240—242° (decomp.); UV $\lambda_{max}^{H_2O}$ (pH 1)[16] 258 mμ (ε 17800); $\lambda_{max}^{H_2O}$ (pH 7)[17] 246 (13400), 263 (sh) (8200); $\lambda_{max}^{H_2O}$ (pH 12)[20] 244 (15000), 267 (sh) (4200); IR ν_{max}^{Nujol} cm^{-1}: 3240, 3100 (NH), 1713 (b, medium, COOH); NMR (DMSO-d_6) τ: 7.77 (3H, s, CH_3), 6.05 (2H, d, J=6 cps, $NHC\underline{H}_2CO_2H$), 3.58 (1H, s, $H_{(5)}$), 2.45 (NH), 1.67 (1H, b, $H_{(2)}$); Mass Spectrum m/e 167 (M^+). *Anal.* Calcd. for $C_7H_9O_2N_3$: C, 50.29; H, 5.43; N, 25.14. Found: C, 50.23; H, 5.56; N, 24.74.

Hydrolysis Studies——Sample solutions of the dipeptides (IIa, b, c, IIIa, b, c, X, and XII) were separately prepared by dissolving them in 0.02N aq. NaOH at 0.01M concentration. Each of the solutions was diluted with a requisite buffer (or aq. HCl or aq. NaOH) of appropriate concentration and H_2O by a factor of 10, and allowed to react under the conditions as specified in Table I. After cooling, the reaction mixture was adequately diluted with H_2O, or, in the case of the reaction at pH 1 or 13, neutralized with aq. NaOH or aq. HCl, and 0.5 ml of the resulting solution was subjected to the colorimetric estimation of the liberated α-amino acid using the photometric ninhydrin method reported by Troll and Cannan.[21] Measurement of the color

19) H. Röhnert, *Arch. Pharm.*, **295**, 697 (1962).
20) Measured in 0.01N aq. NaOH.
21) W. Troll and R.K. Cannan, *J. Biol. Chem.*, **200**, 803 (1953).

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density was effected at 570 mμ with a Hitachi Model 181 spectrophotometer, and concentration of the amino acid was estimated from a calibration curve which had been constructed on an analytical sample. In the cases of IIb and IIc, the following alternative was also adopted: The reaction mixture was concentrated to one fifth of its original volume, and 0.5 ml of the resulting solution was applied along a 13-cm line on Toyo Roshi No. 51 filter paper. Paper electrophoresis was then carried out with a Toyo Kagaku Model EP-2 apparatus for 2 hr at 700 V using 0.01M sodium borate buffer (pH 9). A zone whose mobility was corresponding to that of authentic glycine was excised and extracted with 0.01N aq. HCl (5 ml). A portion (0.5 ml) of the extracts was then used for the determination of glycine as described above.

In the hydrolysis experiments with the tri- and tetrapeptide derivatives (IIId,e,f), approximately 3-ml aliquots of 0.01M solutions (pH 4) of the substrates were separately sealed in small ampoules and allowed to react under the conditions as designated in Table II. For quantitative estimation of the liberated N-(purin-6-yl)amino acids (IVa, b), 0.5 ml of the reaction mixture was applied along a 40-cm line on Toyo Roshi No. 51 filter paper. Then the chromatogram was developed by the ascending method in a solvent system of 1-butanol: H_2O: acetic acid (75: 20: 5, v/v). A zone whose *Rf* value was corresponding to that of authentic IVa or IVb was located under UV rays and was excised and extracted with 0.1N aq. HCl (20 ml). The optical density of the extracts at 275 mμ was then determined, and concentration of IVa or IVb was estimated in the usual manner. The determination of glycine in the reaction mixture was separately carried out by means of the electrophoretic separation followed by the photometric ninhydrin method as described above for IIb and IIc. Detection of other products was accomplished qualitatively by means of paper chromatography, TLC, and paper electrophoresis.

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