Studies of Unusual Amino Acids and Their Peptides. VII. The Syntheses and the Reactions of Iminopeptides[1]

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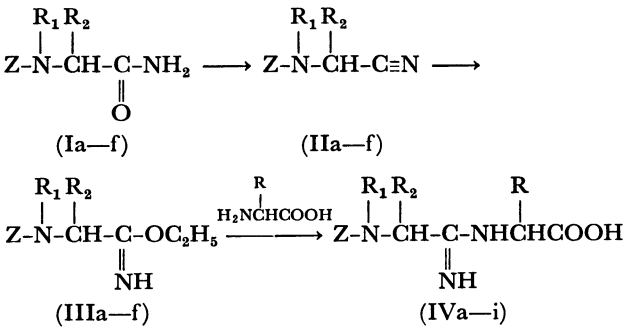
As a basic study for synthesis of bottromycin, the syntheses and the reactions of iminodipeptides were investigated. Nine *N*-benzyloxycarbonyl(Z) iminodipeptides were prepared by the condensation of ethyl Z-amino carboximidates with amino acids according to the method of Ried *et al.* All the imidates were prepared from the corresponding nitriles, and ethyl *N*-Z-2-pyrrolidinecarboximidate (IIIf) was also successfully obtained from Z-proline thioamide by the method of Suydam *et al.* Though Z-(iminoprolyl)glycine was easily deprotected or esterified, its *C*-terminal could not be elongated because it was likely to cyclize into an imidazolone ring. This side reaction could be avoided by locating a carboxyl group further apart from an imino group in a molecule; *i. e.*, the imidate (IIIf) could be coupled with methyl glycylphenylalaninate, thus affording the desired iminotripeptide, Z-ImPro-Gly-Phe-OMe, in a good yield.

Bottromycin, a family of peptide antibiotics produced by *Streptomyces bottropensis*[2] and analogous strains,[3] is an iminopeptide, *i.e.*, a peptide in which an oxo group in the peptide bond is replaced by an imino group. The structure shown in Fig. 1 was given by Umezawa and his co-workers.[4]

An iminopeptide bond is very rare in peptides, though an amidino group itself can be found in amidinomycin,[5] netropsin,[6] and so on.[7] This structural feature of bottromycin has been asserted to contribute to its biological activity to some extent.[8] The present paper will deal with a study of such iminopeptides.

Syntheses of amidines have been widely investigated, but there have been few studies of the syntheses of iminopeptides: Ried and his co-workers[9] synthesized some *N*-benzyloxycarbonyl(Z) iminodipeptides containing optically-active amino acids, and Vargha and Balàzs[10] synthesized *N*-phthalyl(iminoglycyl)-D-valine, along with some analogues. In both cases, however, nothing was reported about their optical properties.

As a basic study of the synthesis of bottromycin, we undertook to investigate the syntheses and the reactions of optically-active iminopeptides, especially those of (iminoprolyl)glycine,[11] which would be an important unit if bottromycin could be conveniently built up by the fragment condensation of three dipeptides (pivaloyl-*t*-leucylvaline,[12] (iminoprolyl)glycine, *β*-methylphenylalanyl-*β*-(2-thiazolyl)-*β*-alanine methyl ester[13]). In principle, we adopted the method of Ried *et al.* for the syntheses of iminopeptides. As an *N*-protecting group, the Z group was chosen because it can easily be removed by hydrogen bromide in anhydrous acetic acid, under which conditions an amidino group seems to be stable. A scheme of the synthesis is shown in Scheme 1.



Scheme 1.

Z-Amino nitriles (II) were prepared by dehydrating the corresponding amides (I) with *p*-toluenesulfonyl chloride in pyridine according to the method of Hirotsu *et al.*[14] The reaction temperature was, however, kept at 50 °C instead of refluxing, and three equivalents of *p*-toluenesulfonyl chloride were used to cover the lowered reaction rate. The excess sulfonyl chloride could easily be removed by stirring the reaction mixture for a few hours with 10% aqueous pyridine at room temperature. Thus, the nitriles were obtained quantitatively in a purer state, hardly any by-products being observed. It is noteworthy that 2-(Z-amino)-3-methylvaleronitrile (IId), which has been reported to solidify only after distillation,[14] could be obtained in crystalline forms without further purification. The results are shown in Table 1.

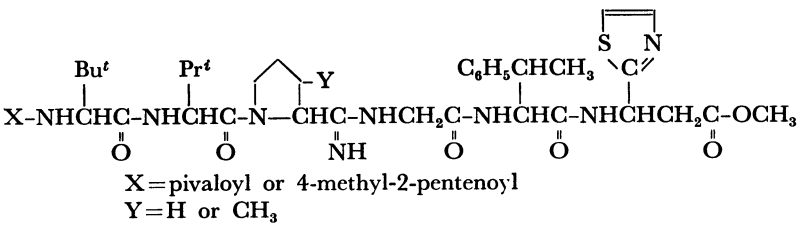


Fig. 1. The structure of bottromycin proposed by Umezawa *et al.*

Table 1. Physical properties of nitriles $\left(\begin{matrix} R_1\ R_2 \\ | \quad | \\ Z\text{-}N\text{-}CH\text{-}C{\equiv}N \end{matrix}\right)$ (IIa—f)

Compd	R_1	R_2	Starting amino acid	Yield (%)	Mp °C	$[\alpha]_D^{23}$ (ethanol)	Molecular formula	Analysis (%) Found (Calcd) C	H	N
IIa	H	H	Gly	99	61—62[a]					
IIb	H	CH_3	Ala	99	82—82.6	−68.9° (c 5.0)[b]				
IIc	H	$(CH_3)_2CH$	Val	100	55.5—56	−61.3° (c 4.5)[c]				
IId	H	$(CH_3)_2CHCH_2$	Leu	99	32—33.3	−53.1° (c 5.0)[d]				
IIe	H	$C_6H_5CH_2$	Phe	99	136—137	−62.4° (c 0.5)	$C_{17}H_{16}N_2O_2$	73.03 (72.84)	5.84 (5.75)	9.92 (9.99)
IIf	$-CH_2CH_2CH_2-$		Pro	99	oil	−87.9° (c 5.0)	$C_{13}H_{14}N_2O_2$	68.17 (67.81)	6.18 (6.13)	12.06 (12.17)

Lit[14]: a) Mp 61—62 °C. b) Mp 84—85.5 °C, $[\alpha]_D^{15}$ −69.1° (c 5.62, ethanol). c) Mp 55—56 °C, $[\alpha]_D^{12}$ −55.2° (c 4.48, ethanol). d) Mp 29.5—32.0 °C, $[\alpha]_D^{24}$ −51.0° (c 5.86, ethanol).

Table 2. Physical properties of *N*-benzyloxycarbonyl-iminodipeptides (IVa—i)

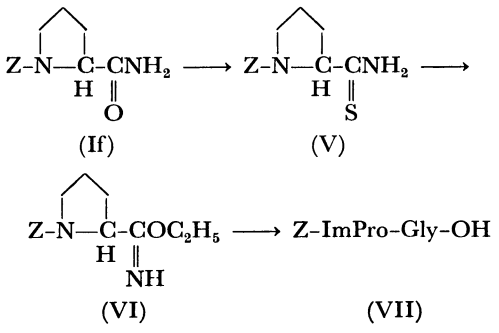
	Compound	Yield (%)	Mp (°C)	$[\alpha]_D$	Molecular formula	Analysis (%) Found (Calcd) C	H	N
IVa	Z-ImGly-Gly-OH	62	245—250 (dec)		$C_{12}H_{15}N_3O_4$	54.18 (54.33)	5.75 (5.70)	15.63 (15.84)
IVb	Z-ImGly-Ala-OH	55	198.5—199.5 (dec)	−27.4°[a]	$C_{13}H_{17}N_3O_4$	55.52 (55.90)	6.32 (6.14)	14.91 (15.05)
IVc	Z-ImGly-Leu-OH	59	178—179 (dec)	−45.7°[b]	$C_{16}H_{23}N_3O_4$	59.61 (59.79)	7.42 (7.21)	13.00 (13.08)
IVd	Z-ImGly-Phe-OH	72	195—196 (dec)	+42.2°[a]	$C_{19}H_{21}N_3O_4$	64.20 (64.21)	6.09 (5.96)	11.72 (11.83)
IVe	Z-ImGly-Pro-OH	73	147—149	−84.9°[b]	$C_{15}H_{19}N_3O_4$	58.57 (59.00)	6.41 (6.27)	13.55 (13.76)
IVf	Z-ImAla-Gly-OH	55	213—215 (dec)	− 2.8°[a]	$C_{13}H_{17}N_3O_4$	55.76 (55.90)	6.29 (6.14)	14.78 (15.05)
IVg	Z-ImLeu-Gly-OH	50	194—196 (dec)	−11.0°[c]	$C_{16}H_{23}N_3O_4 \cdot 1/2H_2O$	58.14 (58.16)	7.14 (7.32)	12.57 (12.72)
IVh	Z-ImPhe-Gly-OH	69	204—206 (dec)	+ 7.4°[a]	$C_{19}H_{21}N_3O_4 \cdot H_2O$	61.05 (61.11)	6.23 (6.21)	11.08 (11.25)
IVi	Z-ImPro-Gly-OH	81	200—201.5 (dec)	−64.4°[b] −65.8°[d]	$C_{15}H_{19}N_3O_4$	58.80 (59.00)	6.29 (6.27)	13.56 (13.76)

a) c 1 in acetic acid at 23 °C. b) c 1 in methanol at 20 °C. c) c 1 in methanol at 25 °C. d) c 1 in ethanol at 28 °C.

Ethyl Z-amino carboximidates (III) were prepared from the nitriles (II) by the method of Pinner.[14,15] Except from glycine and alanine, every free imidate was obtained as an oil and was used in the next reaction without any purification.

Two types of optically-active iminodipeptides (IV) (Z-ImGly-AA-OH and Z-ImAA-Gly-OH)[11] were successfully prepared by the reactions of the imidates (III) with free amino acids in anhydrous methanol under reflux. The results are summarized in Table 2.

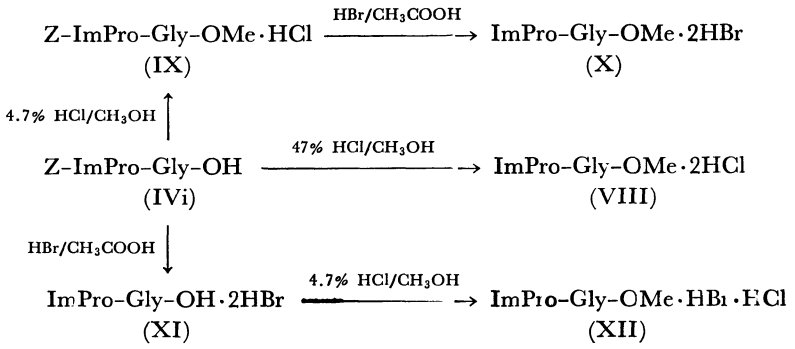
Suydam and his co-workers[16] have reported another convenient method for preparing imidate directly from amide. They obtained a number of aliphatic imidates in fairly good yields by the reaction of the corresponding amides with ethyl chloroformate. Though their method could not be applied to Z-proline amide (If) itself, the corresponding thioamide (V) reacted smoothly with ethyl chloroformate at room temperature, giving an optically-active imidate (VI), which then, by reaction with glycine, afforded Z-(iminoprolyl)-glycine (VII). The optical rotation of this product



Scheme 2.

(VII) agreed well with that of the same compound (IVi) obtained through Pinner's method. (Scheme 2)

Some reactions of iminodipeptides were investigated by using Z-(iminoprolyl)glycine (IVi). When IVi was treated with saturated hydrogen chloride in methanol at room temperature in order to esterify it, the removal of the Z group rapidly occurred, followed by slow esterification, and (iminoprolyl)glycine methyl ester was



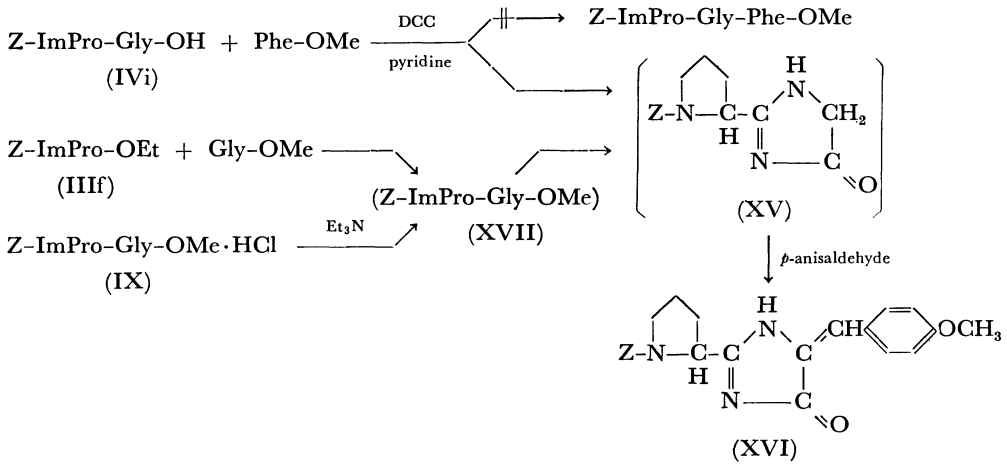
Scheme 3.

obtained as dihydrochloride (VIII). On the other hand, when IVi was treated with 4.7% hydrogen chloride in methanol, it could be quantitatively esterified without the removal of the protecting group and the resulting ester was isolated as monohydrochloride (IX), which was then further treated with hydrogen bromide in acetic acid to give (iminoprolyl)glycine methyl ester dihydrobromide (X). The same ester was also obtained by the esterification of (iminoprolyl)-glycine dihydrobromide (XI) with 4.7% hydrogen chloride in methanol, though the ester was isolated as a hydrochloride-hydrobromide salt (XII). The iminodipeptide (XI) was obtained as expected in the form of dihydrobromide on the treatment of IVi with hydrogen bromide in acetic acid. The correlation between these compounds is shown in Scheme 3.

In order to elongate the *C*-terminal of Z-iminodipeptides (IV), many attempts were made under various conditions, but the expected results were not obtained. One of the troubles is the low solubilities of these compounds in solvents; *e. g.*, Z-(iminoglycyl)glycine (IVa) dissolved neither in acetonitrile, tetrahydrofuran, *N,N*-dimethylformamide, nor dimethyl sulfoxide, though it is fairly soluble in such protic solvents as methanol and acetic acid, and in such basic solvents as pyridine it is soluble to some extent. On the other hand, even a more soluble compound, Z-(iminoprolyl)-glycine (IVi), was hardly soluble in polar aprotic solvents. The use of *N*-isobornyloxycarbonyl(IBOC) derivatives[17] was attempted with hopes founded on their high solubilities:[18] IBOC-proline amide (XIII) and the corresponding nitrile (XIV) were prepared successfully, but the conversion of the nitrile to IBOC-(iminoprolyl)glycine *via* an imidate did not occur smoothly.

We attempted to couple the iminopeptide (IVi) with methyl phenylalaninate in the presence of dicyclohexylcarbodiimide (DCC) in pyridine. The isolated main product, however, was not the desired iminotripeptide derivative, but probably an imidazolone derivative (XV),[19] which might have been produced by the intramolecular cyclization of the starting iminodipeptide, since the same compound was also obtained in a good yield instead of the free iminodipeptide ester (XVII) when the iminodipeptide ester hydrochloride (IX) was treated with triethylamine in methanol. Furthermore, when the imidate (IIIf) was coupled with methyl glycinate, the same compound (XV) was again produced as the main product. Compound XV was too unstable to purify; it was identified by its conversion to a more stable derivative, (*p*-methoxyphenylmethylene)imidazolone (XVI).[20] These facts provide evidence for the smooth cyclization reaction of the iminodipeptide ester (Scheme 4).

From the facts mentioned above, we could not but conclude that it is impossible to elongate the *C*-terminal of an iminodipeptide directly. If an imino group and a carboxyl group were situated further apart in a molecule, such an intramolecular cyclization might be avoided. In order to confirm this possibility, the imidate (IIIf) was coupled with glycylphenylalanine methyl ester hydrobromide in the presence



Scheme 4.

of triethylamine in methanol at room temperature for 2 days. The desired iminotripeptide derivative, *i.e.*, Z-(iminoprolyl)glycylphenylalanine methyl ester (XVIII), was thus successfully obtained in a 61.4% yield after purification by column chromatography. It is interesting that the iminotripeptide ester (XVIII) was isolated as a hydrobromide, though an equimolecular amount of triethylamine was added in order to remove hydrogen bromide.

Consequently, it was concluded that, for the syntheses of bottromycin and its analogues, the route through the fragment condensation of three dipeptides described above is not adequate, while the route through the condensation by the formation of an iminopeptide bond between two tripeptides may be better.

Experimental

All the melting points are uncorrected. The optical rotations were measured by means of a Yanagimoto polarimeter, OR-10. The ORD-curves were recorded on a JASCO ORD/UV-5 spectropolarimeter. The NMR spectra were recorded on a Varian A-60 spectrometer or a Hitachi R-20A spectrometer, using TMS or DSS as the internal standard. Thin-layer chromatographies (TLC) were carried out on Merck's Kieselgel GF_{254} (Type 60). The amino acids and their derivatives mentioned in this report are all of the L-configuration unless otherwise mentioned.

Benzyloxycarbonylamino Acid Amides (I). All the amides were prepared by the mixed anhydride method using ethyl chloroformate.[21] The ammonolysis of the mixed anhydride intermediates was successfully carried out with 28% aqueous ammonia instead of liquid ammonia.

α-Benzyloxycarbonylamino α-Substituted Acetonitriles (II). The nitriles were prepared by the dehydration of the corresponding amides with *p*-toluenesulfonyl chloride in pyridine in the usual way,[14,22] but with some modifications. A typical example was as follows: into a solution of Z-proline amide (12.4 g, 0.15 mol) in dry pyridine, *p*-toluenesulfonyl chloride (28.5 g, 0.15 mol) was stirred at room temperature, and thus the mixture was heated at 50 °C for 4 h. After pyridine had been removed under reduced pressure, the residue was treated with 10% aqueous pyridine (200 ml) with stirring for 2 h. The mixture was extracted with ethyl acetate. The organic layer was washed with 1M-HCl, 1M-$NaHCO_3$, and then water, and dried over $MgSO_4$. The solution was concentrated to afford a pale yellow oil; yield, 11.4 g (99%). The other nitriles, prepared in the same manner, are summarized in Table 1.

α-Benzyloxycarbonylamino Carboximidates (III). The imidates were prepared according to the method of Pinner.[14,15] A typical example was as follows: to a cold solution of *N*-Z-2-cyanopyrrolidine (IIf) (1.15 g, 5 mmol) in a mixture of absolute ethanol (0.3 g, 6.5 mmol) and dry ether (15 ml), dry hydrogen chloride was passed with stirring below −5 °C until the gas was saturated, and then at room temperature for 1 h. The reaction mixture was evaporated under reduced pressure to leave ethyl *N*-Z-2-pyrrolidinecarboximidate hydrochloride as a foamy solid. The imidate hydrochloride was immediately converted to a free imidate in the manner described by Hirotsu *et al.*;[14] a colorless oil of IIIf was thus obtained; yield, 1.375 g (99%); $[\alpha]_D^{21}$ −59° (*c* 1, methanol). All the imidates obtained were used for the next reaction without any purification.

N-Benzyloxycarbonyl Iminodipeptides (IV). General Procedure. A mixture of imidate (0.01 mol) and amino acid (0.012 mol) in dry methanol (50 ml) was refluxed for 3 h. After cooling, the unreacted amino acid was filtered off, and the filtrate was evaporated to dryness. The residual crude iminodipeptide was purified by recrystallization from methanol-ethyl acetate or by column chromatography (silica gel, methanol or methanol-ethyl acetate). The results are summarized in Table 2.

N-Benzyloxycarbonylproline Thioamide (V). This compound was prepared from the corresponding amide (2.48 g, 10 mmol) according to our previously described procedure.[23] The reaction was completed in 2.5 h at room temperature. The recrystallization of the crude product from benzene-petroleum ether gave colorless crystals; yield, 1.78 g (67%); mp 93—94 °C, $[\alpha]_D^{26}$ −49.4° (*c* 1, methanol). Found: C, 59.06; H, 6.24; N, 10.46%. Calcd for $C_{13}H_{16}N_2O_2S$: C, 59.07; H, 6.10; N, 10.60%.

N-Benzyloxycarbonyl(iminoprolyl)glycine (VII), via *Imidate from N-Benzyloxycarbonylproline Thioamide (V).* A mixture of thioamide (V) (529 mg, 2 mmol), ethyl chloroformate (260 mg, 2.4 mmol), and absolute ethanol (0.5 ml) was stirred at room temperature. The reaction proceeded with a smooth evolution of gas and was completed in 2 h. The reaction mixture was then diluted with dry ether to separate a yellow oil. The oil, imidate hydrochloride, was immediately converted to a free imidate, a slightly yellow oil, as described above; yield, 399 mg (65%); $[\alpha]_D^{28}$ −48.9° (*c* 4, methanol).

The imidate, without any purification, was coupled with glycine (150 mg, 2 mmol) in dry methanol under reflux for 3.5 h, giving Z-(iminoprolyl)glycine (222 mg, 56%). $[\alpha]_D^{28}$ −66.9° (*c* 1, ethanol).

(Iminoprolyl)glycine Methyl Ester Dihydrochloride (VIII). To saturated hydrogen chloride in methanol (47%) (1 ml) was added in portions Z-(iminoprolyl)glycine (IVi) (100 mg, 0.33 mmol), which immediately dissolved with the evolution of gas. The solution was allowed to stand at room temperature for 3 days, and then evaporated under reduced pressure to leave white crystals; yield, 80 mg (94.5%); mp 167—168 °C (dec) (methanol-ether), $[\alpha]_D^{20}$ −0.5° (*c* 3, methanol). ORD: $[\phi]_{260}$ +372°, $[\phi]_{300}$ +111°, $[\phi]_{400}$ +23° (*c* 3, methanol). NMR(D_2O): δ 1.9—2.8 (m, 4H, C_3–H and C_4–H in proline), 3.57 (t, J=7 Hz, 2H, C_5–H in proline), 3.81 (s, 3H, CH_3-OCO–), 4.36 (s, 2H, glycine-CH_2). Found: C, 37.05; H, 6.79; N, 16.28%. Calcd for $C_8H_{15}N_3O_2 \cdot 2HCl$: C, 37.22; H, 6.64; N, 16.28%.

N-Benzyloxycarbonyl(iminoprolyl)glycine Methyl Ester Hydrochloride (IX). A solution of IVi (100 mg, 0.33 mmol) in 4.7% hydrogen chloride in methanol (3 ml) was allowed to stand overnight at room temperature. After the solvent had then been removed under reduced pressure, the residue was dissolved in methanol-ethyl acetate and the solution was again evaporated to afford a colorless foamy solid; yield, 115 mg (98.5%); mp 83—87 °C, $[\alpha]_D^{20}$ −55.0° (*c* 0.5, methanol). TLC: R_f 0.18 (methanol-ethyl acetate (1 : 4)). UV: λ_{max}^{MeOH} (ε); 257 nm (197). NMR(D_2O): δ 1.6—2.5 (m, 4H, C_3–H and C_4–H in proline), 3.58 (t, J=7 Hz, 2H, C_5–H in proline), 3.76 (s, 3H, CH_3OCO–), 4.06 (s, 2H, glycine-CH_2), 5.18 (s, 2H, benzyl-CH_2), 7.43 (s, 5H, phenyl). Found: C, 51.34; H, 6.45; N, 11.21%. Calcd for $C_{16}H_{21}N_3O_4 \cdot HCl \cdot H_2O$: C, 51.41; H, 6.47; N, 11.24%.

(Iminoprolyl)glycine Methyl Ester Dihydrobromide (X). The treatment of IX (50 mg) with 25% hydrogen bromide in acetic acid (0.15 g) at room temperature for 45 min gave (iminoprolyl)glycine methyl ester dihydrobromide; yield, 41 mg (84%); mp 165—166 °C (dec) (methanol-ether), $[\alpha]_D^{17}$ −2.0° (*c* 1, methanol). ORD: $[\phi]_{250}$ +510°, $[\phi]_{300}$ +101°, $[\phi]_{400}$ +14° (*c* 1, methanol). Found: C, 27.44; H, 5.12;

N, 11.94%. Calcd for $C_8H_{15}N_3O_2 \cdot 2HBr$: C, 27.69; H, 4.94; N, 12.11%.

(Iminoprolyl)glycine Dihydrobromide (XI). This compound was obtained by the removal of the Z group from IVi (100 mg, 0.33 mmol) with 25% hydrogen bromide in acetic acid (340 mg) as usual; yield, 100 mg (92%); mp 161—162 °C (methanol-ether), $[\alpha]_D^{20}$ −9.2° (*c* 0.4, methanol). Found: C, 25.65; H, 4.67; N, 12.28%. Calcd for $C_7H_{13}N_3O_2 \cdot 2HBr$: C, 25.25; H, 4.54; N, 12.62%.

(Iminoprolyl)glycine Methyl Ester Hydrochloride-hydrobromide (XII). The treatment of XI (60 mg) with 4.7% hydrogen chloride in methanol (3 ml) at room temperature for 4 days gave white crystals; yield, 39 mg (84%); mp 164—165 °C (dec) (methanol-ether). Found: C, 31.41; H, 5.35; N, 14.65%. Calcd for $C_8H_{15}N_3O_2 \cdot HBr \cdot HCl$: C, 31.75; H, 5.66; N, 13.89%.

N-*Isobornyloxycarbonylproline.* This compound was prepared in the manner described by Fujino and his co-workers;[17] yield, 57%; mp 134—135 °C (lit,[17] mp 135.5—136 °C).

N-*Isobornyloxycarbonylproline Amide (XIII).* The mixed anhydride method described above for Z-amino acid amides gave the desired amide (65.4%), along with the recovered starting material (30.6%). The recrystallization of the amide from aqueous ethanol afforded white crystals; mp 175.5—176 °C, $[\alpha]_D^{28}$ −83.7° (*c* 1, ethanol). Found: C, 65.14; H, 9.26; N, 9.41%. Calcd for $C_{16}H_{26}N_2O_3$: C, 65.28; H, 8.90; N, 9.52%.

N-*Isobornyloxycarbonyl-2-cyanopyrrolidine (XIV).* IBOC-proline amide (XIII) (3.94 g, 0.01 mol) was dehydrated with *p*-toluenesulfonyl chloride (5.7 g, 0.03 mol) in pyridine (20 ml) in the manner described above for the Z series, giving colorless crystals; yield, 3.70 g (98.7%); mp 73—76 °C.

The analytical sample was obtained by recrystallization from petroleum ether; mp 78—79 °C, $[\alpha]_D^{28}$ −127.1° (*c* 1, ethanol). Found: C, 69.39; H, 9.00; N, 10.20%. Calcd for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14%.

Formation of the Imidazolone Derivative (XV). a) By the Reaction of N-*Benzyloxycarbonyl(iminoprolyl)glycine (IVi) with Methyl Phenylalaninate in the Presence of Dicyclohexylcarbodiimide*: Into a solution of IVi (153 mg, 0.5 mmol), methyl phenylalaninate hydrochloride (113 mg, 0.5 mmol), and triethylamine (51 mg, 0.5 mmol) in pyridine (5 ml), DCC (103 mg, 0.5 mmol) was stirred at room temperature. After 1 day, the resulting white crystals were filtered off and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a preparative thin layer with methanol–ethyl acetate (1 : 9) to afford an oil; yield, 18 mg (19%); TLC: R_f 0.37 (methanol–ethyl acetate (1 : 9)).

b) By the Treatment of N-*Benzyloxycarbonyl(iminoprolyl)glycine Methyl Ester Hydrochloride (IX) with Triethylamine*: A solution of the hydrochloride (IX) (100 mg) and triethylamine (60 mg) in methanol (3 ml) was allowed to stand at room temperature for 3 h. The solution was then evaporated under reduced pressure to give a brown-yellow oil, which was chromatographed on a preparative thin layer; yield, 62 mg (77%); TLC: R_f 0.37 (methanol–ethyl acetate (1 : 9)).

c) By the Reaction of Ethyl N-*Benzyloxycarbonyl-2-pyrrolidinecarboximidate (IIIf) with Methyl Glycinate*: A mixture of the imidate (IIIf) (2.36 g, 8.5 mmol), methyl glycinate hydrochloride (0.76 g, 8.5 mmol), and triethylamine (0.86 g, 8.5 mmol) in dry tetrahydrofuran (50 ml) was stirred at room temperature for 2 days. After a white precipitate had been filtered off, the filtrate was concentrated to give a reddish brown oil. The oil was chromatographed on a silica gel column with methanol–ethyl acetate (1 : 4), thus affording as the main product a yellow oil, which soon turned a reddish brown; yield, 0.82 g (29%); TLC: R_f 0.37 (methanol–ethyl acetate (1 : 9)).

*2-(*N-*Benzyloxycarbonyl-2-pyrrolidinyl)-5(or 4)-(*p-*methoxyphenylmethylene)-2-imidazolin-4(or 5)-one (XVI).* a) A solution of the crude compound (XV) (100 mg) and *p*-anisaldehyde (360 mg) in methanol (1 ml) was allowed to stand at room temperature for 2 days. The reaction mixture was then chromatographed on a preparative thin layer with benzene–ethyl acetate (3 : 2) to give two sorts of yellow crystals; major product; yield, 43 mg; TLC: R_f 0.62 (benzene–ethyl acetate (2 : 3)); minor product: yield, 11 mg; TLC: R_f 0.44. The analytical sample of the major product was obtained by recrystallization from ethyl acetate; mp 194—195 °C, $[\alpha]_D^{20}$ −53.1° (*c* 1, $CHCl_3$). UV: $\lambda_{max}^{CHCl_3}$ (ε); 368 nm (3.70×10^4). NMR ($CDCl_3$): δ 1.8—2.5 (m, 4H, C_3–H and C_4–H in pyrrolidine ring), 3.58 (t, J=6 Hz, 2H, C_5–H in pyrrolidine ring), 3.86 (s, 3H, OCH_3), 4.7—5.0 (m, 1H, C_2–H in pyrrolidine ring), 5.19 (s, 2H, benzyl-CH_2), 6.95 (d, J=9 Hz, 2H, *meta*-H of *p*-methoxyphenyl), 7.09 (s, 1H, C=C<u>H</u>), 7.35 (s, 5H, C_6H_5–), 8.13 (d, J=9 Hz, 2H, *ortho*-H of *p*-methoxyphenyl), 9.36 (s, 1H, NH). Found: C, 68.06; H, 5.72; N, 10.05%. Calcd for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36%.

The minor product seemed to be an isomer since it gradually changed into the major product in methanol, but it was not investigated further.

b) A more convenient method to prepare XVI was as follows: a mixture of IX (608 mg), *p*-anisaldehyde (2.23 g), and triethylamine (331 mg) in methanol (2 ml) was allowed to stand at room temperature for 2 days. After the solvent had been removed under reduced pressure, the residue was taken up in ethyl acetate in order to remove triethylamine hydrochloride. The solution was then concentrated to give a yellow oil, which was chromatographed on a silica gel column with benzene–ethyl acetate (4 : 1—3 : 2), affording yellow crystals; yield, 507 mg (77%); mp 193—194 °C, TLC: R_f 0.62 (benzene–ethyl acetate (2 : 3)).

Glycylphenylalanine Methyl Ester Hydrobromide. This compound was prepared by the coupling of Z-glycine with methyl phenylalaninate using DCC, followed by the removal of the Z group with 25% hydrogen bromide in acetic acid, according to the method of Mazur and Schlatter;[24] yield, 94%; mp 166—168 °C (dec) (lit,[24] mp 167—170 °C). It was used for the next reaction without further purification.

N-*Benzyloxycarbonyl(iminoprolyl)glycylphenylalanine Methyl Ester (XVIII).* A solution of the imidate (IIIf) (665 mg, 2.4 mmol), glycylphenylalanine methyl ester hydrobromide (634 mg, 2.0 mmol), and triethylamine (220 mg, 2.0 mmol) in dry methanol (10 ml) was stirred at room temperature for 1 day. The solution was then evaporated under reduced pressure to leave a syrup with some crystals. The syrup was taken up in ethyl acetate, and any insoluble materials were filtered off. The filtrate was evaporated under reduced pressure, and the residue was chromatographed on a silica gel column with methanol–ethyl acetate (1 : 9), giving a foamy solid; yield, 683 mg (61.4%); mp 90—94 °C, $[\alpha]_D^{23}$ −23.6° (*c* 0.75, methanol). Found: C, 53.94; H, 5.84; N, 10.03%. Calcd for $C_{25}H_{30}N_4O_5 \cdot HBr \cdot 1/2H_2O$: C, 53.96; H, 5.80; N, 10.07%.

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7) These peptides should be classified as peptide amidines instead of as iminopeptides, since they contain an amidino group in the terminal of the molecule, not between amino acid residues in peptide.

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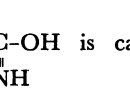
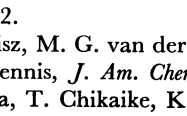
11) The following nomenclatures and abbreviations are used for iminopeptides. An amino acid containing =NH instead of =O in the carboxyl group is called an imino amino acid and abbreviated as ImAA (AA=amino acid); *e.g.*,  is called iminoproline (ImPro). Therefore,  is called (iminoprolyl)glycine (ImPro-Gly), and ethyl Z-amino carboximidate (III) may be abbreviated as Z–ImAA–OEt.

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