after washing with H_2O , was evaporated to dryness. Addition of AcOEt to the residue formed a solid, which was recrystallized from MeOH and AcOEt ; yield 0.16 g (71%). $[\alpha]_D^{25} - 67.0^\circ$ ($c=1.0$, MeOH). Identity of this product and the authentic sample of N^α -benzyloxycarbonylprolylprolyl- N^ϵ -formyllysylaspartic acid²²⁾ was established by comparison of their R_f values (both 0.53, by the iodine stain) and their IR spectra.

Reaction of a Mixed Anhydride of N^α -Benzyloxycarbonyl- γ -benzylglutamate with Histidylphenylalanylarginyl Tryptophylglycine—A mixed anhydride, prepared from N^α -benzyloxycarbonyl- γ -benzylglutamate (0.22 g) in tetrahydrofuran (6 ml) with triethylamine (0.08 ml) and ethyl chloroformate (0.07 ml) was added to a solution of histidylphenylalanylarginyltryptophylglycine acetate³²⁾ (0.17 g) and triethylamine (0.03 ml) in dimethylformamide (6 ml) and the solution was stirred in an ice-bath for 3 hr. The solvent was evaporated and the residue was treated with AcOEt . The resulting powder was washed successively with AcOEt and H_2O ; yield 0.15 g. R_f 0.77 and 0.53; both ninhydrin negative but Pauly, Sakaguchi and Ehrlich positive spots. This powder in 20% AcOH (25 ml) was hydrogenated over a Pd catalyst at room temperature for 7 hr. During the hydrogenation, the spot of R_f 0.53 remained unchanged while the spot of R_f 0.77 disappeared and new ninhydrin positive spot of R_f 0.33 was detected. The catalyst was removed by filtration and the filtrate was evaporated. The residue in H_2O (150 ml) was applied to a column of CM-cellulose (2×13 cm), which was eluted with 0.05M pyridine acetate buffer at pH 5.0. Absorbancy at 280 $m\mu$ was determined in each fraction (20 ml each). Two peaks were detected and the solvents of each peak were evaporated to dryness and the residues were lyophilized respectively; yield of the front peak (tube No. 55 to 75), 0.023 g (12%), $[\alpha]_D^{25} - 21.0^\circ$ ($c=0.5$, 1N AcOH), R_f 0.53 ninhydrin negative but Pauly, Sakaguchi and Ehrlich positive spot. Amino acid ratios in an acid hydrolysate $\text{His}_{0.97}\text{Phe}_{1.00}\text{Arg}_{0.96}\text{Gly}_{1.05}$ (average recovery 85%). Yield of the behind peak (tube No. 90 to 118), 0.065 g (32%), $[\alpha]_D^{25} - 13.5^\circ$ ($c=0.6$, 1N AcOH), ($c=\text{lit.}^{36})$ $[\alpha]_D - 15^\circ$ in 1N AcOH , lit.³⁷⁾ $[\alpha]_D^{25} - 17.3^\circ$ in AcOH , lit.³⁸⁾ -18.0° in 1N AcOH). R_f 0.33, single spot positive to ninhydrin, Pauly, Sakaguchi and Ehrlich test. Amino acid ratios in an acid hydrolysate $\text{Glu}_{1.07}\text{His}_{1.11}\text{Phe}_{0.94}\text{Arg}_{1.00}\text{Gly}_{0.99}$ (average recovery 86%).

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Studies on Peptides. XXV.¹⁾ A Convenient Procedure for the Preparation of *p*-Methoxybenzyl Azidoformate

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Recently we described a convenient procedure for the preparation of *tert*-butyl azidoformate, which involved the direct reaction of *tert*-butyl chloroformate and hydrazoic acid in the presence of a base.³⁾ We have now found that this procedure, with slight modification,

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could be successfully applied to the preparation of *p*-methoxybenzyl azidoformate, an important reagent for the synthesis of *p*-methoxybenzyloxycarbonylamino acids.

Because of the ease of removal by mild acidolysis, the *p*-methoxybenzyloxycarbonyl group, introduced by McKay and Albertson,⁴ proved to be useful in peptide synthesis, like *tert*-butoxycarbonyl group, as a reversible protecting group for the amino function in peptides and amino acids.⁵ *p*-Methoxybenzyloxycarbonylamino acids were synthesized initially by the reaction of isocyanocarboxylic acid esters with *p*-methoxybenzyl alcohol.⁴ Later *p*-methoxybenzyl *p*-nitrophenyl carbonate⁶ or *p*-methoxybenzyl *N*-hydroxypiperidyl carbonate⁷ or *p*-methoxybenzyloxycarbonyl fluoride⁸ was employed for this purpose. These compounds are commonly prepared during the synthesis analogous to that of *tert*-butoxycarbonylamino acids using *p*-methoxybenzyl azidoformate.⁶ However, this procedure requires multiple steps of reaction *via*, phenyl chloroformate, *p*-methoxybenzylphenyl carbonate and *p*-methoxybenzyloxycarbonyl hydrazine.⁹ This is due to instability of *p*-methoxybenzyl chloroformate at room temperature. Taking advantage of the fact that the chloroformate is rather stable to hydrolysis in cold, Sofuku, *et al.*¹⁰ and Honda, *et al.*¹¹ succeeded independently in the direct preparation of the hydrazide mentioned above and *p*-methoxybenzyloxycarbonylamino acids by the direct reaction of *p*-methoxybenzyl chloroformate with hydrazine or amino acids in an alkaline solution.

In our present studies, we allowed *p*-methoxybenzyl chloroformate and sodium azide to react in the presence of pyridine and obtained *p*-methoxybenzyl azidoformate in nearly quantitative yield. The needle crystalline compound obtained in the present method melts at 32° as recorded by Weygand and Hunger⁶ and exhibited the identical IR spectra with the authentic sample prepared by the known method.^{10,11} The azide can not be distilled without decomposition but can be stored in cold for several months as recorded.⁶ This property permits *p*-methoxybenzyl azidoformate to serve as a direct reagent for the preparation of *p*-methoxybenzyloxycarbonylamino acids *in situ* and our present procedure appears to have reduced some disadvantage suffered in the preparation of these amino acid derivatives.

***p*-Methoxybenzyl Azidoformate**

The entire reaction was carried out at -5° in an ice-NaCl bath. An ice-cooled solution of *p*-methoxybenzyl alcohol (138 g) in anhydrous ether (375 ml) was added with stirring to a solution of phosgen (120 g) in anhydrous ether (1000 ml) over a period of 30 min. Stirring was continued for 30 min and then slow stream of dry nitrogen was passed through the solution for 30 min in order to remove the excess of phosgen and hydrogen chloride. To the solution containing *p*-methoxybenzyl chloroformate, a mixture of sodium azide (195 g) in water (500 ml) and pyridine (85 ml) was added with vigorous stirring for 1 hr. Stirring was further continued for 30 min. The ether layer was separated, washed with 10% citric acid and 1*N* NaHCO₃, dried over Na₂SO₄ and evaporated in vacuo at 15°. Petroleum ether (bp 35–70°) was added to the residue, which was kept on standing in a refrigerator to give needles; yield 194 g (94%), mp 32° (lit.⁶ 32°). *Anal.* Calcd. for C₉H₉O₃N₃: C, 52.17; H, 4.38; N, 20.28.

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Found: C, 52.25; H, 4.20; N, 20.44. IR spectra: $-\text{CON}_2$ 2140 cm^{-1} and $-\text{O}-\text{CO}-$ 1727 cm^{-1} . Identity of this product with the sample prepared through the hydrazide was established by comparison of their IR spectra.

Note 1. Progress of the reaction was followed by thin-layer chromatography on silica gel (Kieselgel G Merck) in the solvent system of benzene as recorded by Honda, *et al.*¹¹⁾ Ceric sulfate was used to locate the compounds. The red brown color was observed when a solution of ceric sulfate (1 g) in 10% sulfuric acid (100 ml) was sprayed on a chromatographic plate, which was heated with the gentle flame on a asbestos sheet, as for the detection of alkaloids and terpenes: *p*-methoxybenzyl alcohol *Rf* 0.03; *p*-methoxybenzyl chloroformate *Rf* 0.61; *p*-methoxybenzyl azidoformate *Rf* 0.40.

Note 2. When a large excess of phosgen was employed, formation of some unidentified product (*Rf* 0.55, IR 1700 cm^{-1}) was noted. This can be separated easily since it remained in the mother liquid of the desired crystalline azidoformate.

Note 3. Pyridine of the above procedure can be substituted with triethylamine; yield 82%.

Note 4. The use of hydrazoic acid and pyridine, instead of sodium azide, gave the azidoformate largely contaminated with the unidentified product mentioned in note 2.

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Inhibition of the Copper-catalyzed Oxidation of Ascorbic Acid by 1,10-Phenanthroline

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In the course of the copper-catalyzed oxidation of ascorbic acid, copper ion is turned over, depending probably on the redox potential, between cupric and cuprous ions. The turn-over of copper ion, which is expected to control the rate of the oxidation, may be expressed briefly as follows;

where AH^- and $\cdot\text{AH}$ represent the monoionic ascorbate anion and the ascorbate free radical, respectively. The complexation of copper ion, which interferes either step (1) or (2), retards generally the oxidation.²⁾ In the previous paper, it was elucidated that the inhibition by inorganic anions was partly due to the complex formation between cupric ion and the inorganic anion, by which the interaction of copper ion to the ascorbate anion to form an active inter-

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