An Improved Synthesis of Gramicidin S *via* Solid-Phase Synthesis and Cyclization by the Azide Method

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Abstract: An improved method is described for the preparation of the antibiotic gramicidin S, homodetic, cyclic decapeptide. The open-chain, protected decapeptide was synthesized on a polymer support, starting with leucine as the C-terminal amino acid residue, and was cleaved quantitatively by hydrazinolysis. The α-amino protecting *p*-methoxybenzyloxycarbonyl group was removed with acid and the resulting decapeptide hydrazide was converted to the azide with nitrous acid. Cyclization of the azide in pyridine gave the di-*p*-nitrobenzyloxycarbonyl-substituted cyclic decapeptide in 85% yield. The purity of the peptide derivatives was evaluated spectrophotometrically on the basis of the absorptivity at 268 nm of δ-*p*-nitrobenzyloxycarbonylornithine. Successive catalytic hydrogenation and fractional crystallization produced the synthetic gramicidin S dihydrochloride in 50–60% yield (overall yield 43–51%). The synthetic product exhibited the same antibacterial activity as that of natural gramicidin S.

The usefulness of solid-phase synthesis has been demonstrated for a number of biologically active peptide hormones. This may have been seen, for example, in the syntheses of bradykinin,[1] bradykininyl-bradykinin,[2] and [L-Ala^3,L-Ile^5]-angiotensin II.[3] The solid-phase technique is most reliable for the synthesis of polypeptide chains of 10–20 amino acid residues. Longer peptide products have proved to be heterogeneous mixtures, due to slightly inefficient coupling at each step,[4] requiring special techniques for purification, such as affinity chromatography.[5]

Recently, gramicidin S, one of the cyclic polypeptide antibiotics produced by *Bacillus brevis*, was prepared *via* solid-phase synthesis of the open-chain decapeptide sequence.[6,7] The linear peptides were cleaved from the supporting resin with HBr in trifluoroacetic acid and cyclized using *N,N'*-dicyclohexylcarbodiimide as the condensing reagent. A potent analog, [Gly^5,Gly^{10}]-gramicidin S,[8] has been synthesized by the same principle.[9] Overall yields have been much better than those obtained by conventional liquid-phase synthesis.[10]

We report here an improved method of preparation of gramicidin S, starting with solid-phase synthesis of the open-chain decapeptide. The protected peptide was cleaved from the supporting resin by hydrazinolysis,[11] and the cyclization reaction was carried out by the azide method. Cyclization by the azide procedure of a peptide as long as ten residues has not been reported before.

The *p*-methoxybenzyloxycarbonyl group[12] used as the α-amino protecting group is easily cleaved with 1 *N* HCl in acetic acid without damage to the *p*-nitrobenzyloxycarbonyl group.[13] Anisole was added to the acetic acid to trap undesirable *p*-methoxybenzyl cations released. The ultraviolet spectrum of the *p*-methoxybenzyloxycarbonyl group (dicyclohexylammonium *p*-methoxybenzyloxycarbonylprolinate in ethanol) showed an absorption maximum at 275 nm (ϵ 1.40 $\times$ 10^3) with a shoulder at 281 nm (ϵ 1.20 $\times$ 10^3) and extinction at 268 nm of 1.08 $\times$ 10^3. The δ-amino group of ornithine was protected by the *p*-nitrobenzyloxycarbonyl group[13] which has a strong absorption band at 268 nm (ϵ 1.01 $\times$ 10^4 in ethanol, as dicyclohexylammonium *p*-nitrobenzyloxycarbonylglycinate) and which is very resistant to acid but easily removed by catalytic hydrogenation. Purity of the synthetic intermediates was monitored spectrophotometrically on the basis of absorption at 268 nm and of amino acid analysis of the hydrolysates.

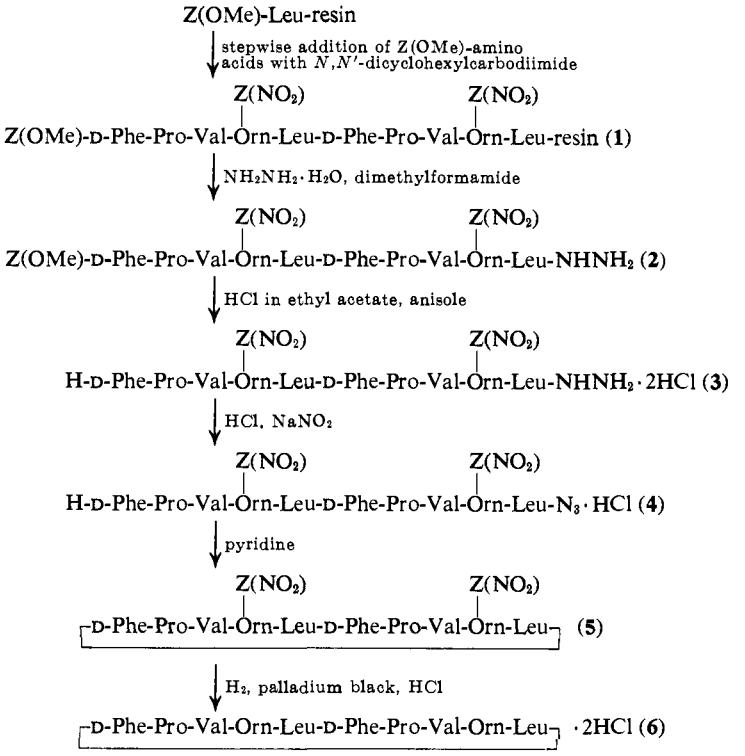
The outline of the present synthesis is given in Scheme I. The protected linear decapeptide sequence of gramicidin S was built up by stepwise addition of *p*-methoxybenzyloxycarbonyl amino acids to the Merrifield resin, starting with leucine as the C-terminal amino acid.

Hydrazinolytic cleavage of the protected decapeptide hydrazide **2** from the resin support was carried out as described,[11] except for the use of 100% hydrazine hydrate. Cleavage was complete, as judged from the absence of detectable amino acids in the hydrolysate of the resin. The cleaved, protected decapeptide hydrazide **2** was estimated to be 98% pure on the basis of its absorption at 268 nm due to the *p*-nitrobenzyloxycarbonyl and *p*-methoxybenzyloxycarbonyl chromophores involved.

The *p*-methoxybenzyloxycarbonyl group of **2** was removed in ethyl acetate containing hydrogen chloride in the presence of anisole, and the resulting decapeptide hydrazide **3** was converted to the azide **4** with nitrous acid. The azide solution was introduced directly into a larger volume of pyridine kept at 0°, and the reaction mixture was stirred for 2 days to allow cyclization. The crude cyclized product (94% yield) obtained was further purified by ion-exchange chromatography on Dowex-50

(1) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).
(2) V. A. Najjar and R. B. Merrifield, *ibid.*, **5**, 3765 (1966).
(3) M. C. Khosla, R. R. Smeby, and M. F. Bumpus, *ibid.*, **7**, 754 (1967).
(4) C. B. Anfinsen, D. A. Ontjes, M. Ohno, L. Corley, and A. Eastlake, *Proc. Nat. Acad. Sci. U. S.*, **58**, 1806 (1967).
(5) D. A. Ontjes and C. B. Anfinsen, *J. Biol. Chem.*, **244**, 6316 (1969).
(6) H. Klostermeyer, *Chem. Ber.*, **101**, 2823 (1968).
(7) G. Losse and K. Neubert, *Tetrahedron Lett.*, 1267 (1970).
(8) H. Aoyagi, T. Kato, M. Ohno, M. Kondo, and N. Izumiya, *J. Amer. Chem. Soc.*, **86**, 5700 (1964); H. Aoyagi, T. Kato, M. Ohno, M. Kondo, M. Waki, and N. Izumiya, *Bull. Chem. Soc. Jap.*, **38**, 2139 (1965).
(9) J. Halstrom and H. Klostermeyer, *Justus Liebigs Ann. Chem.*, **715**, 208 (1968).
(10) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **40**, 624 (1957).
(11) M. Ohno and C. B. Anfinsen, *J. Amer. Chem. Soc.*, **89**, 5994 (1967).
(12) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).
(13) F. H. Carpenter and D. T. Gish, *J. Amer. Chem. Soc.*, **74**, 3818 (1952).

Scheme I. Scheme for Synthesis of Gramicidin S[a]



[a] Prefix L for L-amino acid residues is omitted. Abbreviations used are: Z(OMe), *p*-methoxybenzyloxycarbonyl; Z(NO2), *p*-nitrobenzyloxycarbonyl.

(H^+ form). The overall yield for cyclization was 85%. The molecular weight of the product, measured by vapor pressure osmometry, was 1492, in good agreement with the calculated value for the di-*p*-nitrobenzyloxycarbonyl-substituted cyclic decapeptide (**5**), 1499.7.

Catalytic hydrogenation of **5** in methanol containing hydrogen chloride gave a mixture of the deprotected, cyclic peptide dihydrochloride (**6**) and *p*-toluidine hydrochloride, derived from the *p*-nitrobenzyl moiety of the *p*-nitrobenzyloxycarbonyl group. Separation of **6** from *p*-toluidine hydrochloride was achieved simply by adding 1 *N* hydrochloric acid to the syrup (see Experimental Section). Yields of **6** in repeated experiments have ranged between 50 and 60% after recrystallization. The antibacterial activity of **6** was essentially the same as that of natural gramicidin S (see Table I).

Table I. Inhibitory Activity of Synthetic Peptide **6** and Natural Gramicidin S on Microorganisms[a]

	Minimum inhibitory concn, μg/ml[b]	
	Gramicidin S	Synthetic peptide **6**
Staphylococcus aureus 209 P	0.78	0.78
Staphylococcus aureus Aritomi	3.18	3.18
Bacillus subtilis ATCC 6623	0.78	0.78
Escherichia coli IAM 1253	25	25
Proteus vulgaris IAM 1025	>100	>100
Pseudomonas aeruginosa IAM 1007	50	>100
Shigella sonnei 191-66	25	25
Candida albicans IAM 4888	6.25	6.25

[a] Hert infusion broth medium (Difco) was used as an assay medium, except for the use of Sabourauds medium for *C. albicans*. [b] Minimum concentrations necessary for complete inhibition of growth were judged after incubation for 24 hr at 37° except for *C. albicans*, which was incubated for 24 hr at 27°.

Advantages of the present synthetic method for gramicidin S may be ascribed to (a) quantitative detachment of the protected peptide as its hydrazide by hydrazinolysis, (b) an excellent yield of cyclization reaction by the azide procedure, resulting in an overall yield of 43–51%, and (c) spectrophotometric monitoring of purity of the intermediates based on the strong absorption of the *p*-nitrobenzyloxycarbonyl chromophore at 268 nm. This synthetic method may be useful for studies of the relationship between the chemical structure and biological activity of gramicidin S as well as a number of other homodetic cyclic peptide antibiotics.

Experimental Section

Melting points are uncorrected. Ultraviolet spectra were taken with a Hitachi spectrophotometer, Model EPS-3T. Optical rotations were measured with a Yanagimoto polarimeter, OR-20. Molecular weight was estimated with a Hitachi-Perkin-Elmer vapor pressure osmometer, Model 115. Amino acid analyses of peptide derivatives were carried out on samples that had been hydrolyzed with constant boiling hydrochloric acid for 48 hr in evacuated, sealed tubes at 110°, and were recorded on a Hitachi amino acid analyzer, KLA-3A. Thin-layer chromatography was performed on Merck silica gel G plates using chloroform–methanol (5:1 v/v) as the developing solvent. Paper electrophoresis was carried out under the following conditions: paper, Toyo Roshi No. 52 chromatography paper; solvent, formic acid–acetic acid–methanol–water (1:3:6:10 v/v), pH 1.8; voltage gradient, 17 V/cm. Substances bearing a free amino group were detected by spraying ninhydrin followed by heating, and those of amino group blocked substances by spraying 47% hydrobromic acid followed by heating and then ninhydrin.

p-Nitrobenzyloxycarbonylglycine was prepared from *p*-nitrobenzyloxycarbonyl chloride[13] and glycine by the Schotten–Baumann procedure, and the acid was converted to its dicyclohexylammonium salt (mp 171–172°). *p*-Methoxybenzyloxycarbonyl amino acids of leucine, valine, proline, and D-phenylalanine were prepared by the reaction of each amino acid with *p*-methoxybenzyloxycarbonyl azide[12] or chloride.[14] α-*p*-Methoxybenzyloxycarbonyl-δ-*p*-nitro-

benzyloxycarbonylornithine was prepared as described below. All *p*-methoxybenzyloxycarbonyl amino acids were converted to the crystalline dicyclohexylammonium salts which were recrystallized with appropriate solvents. Before use for coupling, *p*-methoxybenzyloxycarbonyl amino acids were generated from the dicyclohexylammonium salts as follows. A suspension of the salt (6.5–9 mmol) in 60 ml of ethyl acetate was shaken with 40 ml of 1 *N* citric acid in a separatory funnel, and the ethyl acetate layer was washed twice with 40-ml portions of fresh 1 *N* citric acid and once with water, dried over Na_2SO_4, and evaporated. The resulting colorless oil was used directly for the coupling reaction.

δ-*p*-Nitrobenzyloxycarbonylornithine. To 100 ml of boiling water containing ornithine monohydrochloride (14.34 g, 85 mmol), basic copper carbonate (*ca.* 10 g) was added in portions. The hot suspension was filtered to remove excess copper carbonate and washed with 40 ml of hot water. The combined filtrates were cooled in an ice bath. To this solution, a solution of *p*-nitrobenzyloxycarbonyl chloride (22 g, 0.1 mol) in 50 ml of dioxane and 120 ml of 1 *N* sodium hydroxide was added in four portions over 30 min with mechanical stirring and continuous cooling. Stirring was continued for a further 1.5 hr at room temperature. The precipitated solid was then filtered and washed with water, acetone, and ether, giving 22.5 g (94%) of product. The faint blue copper complex was suspended in 1 *N* hydrochloric acid (200 ml) and treated with hydrogen sulfide for 1 hr. The copper sulfide precipitate was filtered off and washed with water. The combined filtrates were neutralized with aqueous ammonia (pH 6), giving a precipitate which was filtered and recrystallized from a larger volume of boiling water. The crystalline product was dried *in vacuo:* 15 g (61%); mp 231–232° dec; $[\alpha]^{25}D$ +14° (*c* 0.675, 6 *N* HCl).

Anal. Calcd for $C_{13}H_{17}O_6N_3$: C, 50.16; H, 5.50; N, 13.50. Found: C, 49.87; H, 5.57; N, 13.18.

α-*p*-Methoxybenzyloxycarbonyl-δ-*p*-nitrobenzyloxycarbonylornithine and Its Dicyclohexylammonium Salt. To an ice-cooled solution of δ-*p*-nitrobenzyloxycarbonylornithine (4.36 g, 14 mmol) dissolved in 15 ml of 1 *N* sodium hydroxide, an ethereal solution (8 ml) of *p*-methoxybenzyloxycarbonyl chloride (16 mmol) and 16 ml of 1 *N* sodium hydroxide were added in four portions over 30 min with vigorous stirring. Stirring was continued for 1.5 hr at room temperature, and the mixture was shaken with additional fresh ether. An aqueous phase was separated and acidified with 0.5 *M* citric acid to pH 3.5. The oil obtained was extracted with three 30-ml portions of ethyl acetate. The combined extracts were washed with water, dried over Na_2SO_4, and evaporated to give a colorless oil.

A small portion of the oil was crystallized by adding ether and petroleum ether and by standing overnight in a refrigerator. Recrystallization from methanol–ether–petroleum ether gave 0.40 g of crystals; mp 122–124°; $[\alpha]^{25}D$ −4° (*c* 1.79, methanol).

Anal. Calcd for $C_{22}H_{25}O_9N_3$: C, 55.57; H, 5.30; N, 8.84. Found: C, 55.64; H, 5.48; N, 8.93.

The residual oil was dissolved in a small volume of ethyl acetate and dicyclohexylamine (2.5 ml) was added followed by a mixture of ether and petroleum ether (1:1). The crystalline dicyclohexylammonium salt was filtered and recrystallized from ethanol–ether, 6.74 g (overall yield 80%); mp 128–130°; $[\alpha]^{25}D$ +3.2° (*c* 1.45, methanol).

Anal. Calcd for $C_{34}H_{48}O_9N_4$: C, 61.72; H, 7.37; N, 8.53. Found: C, 61.44; H, 7.52; N, 8.44.

***p*-Methoxybenzyloxycarbonylleucyl-Resin.** To 5.4 g of chloromethylated copolystyrene–divinylbenzene (2%) resin (1.47 mmol of Cl/g) obtained from the Institute for Protein Research of Osaka University were added 35 ml of anhydrous ethanol containing *p*-methoxybenzyloxycarbonylleucine, which had been generated from 3.40 g (7 mmol) of the dicyclohexylammonium salt, and 1.0 ml (7 mmol) of triethylamine. The mixture was refluxed for 72 hr with continuous stirring. The resin was filtered, rinsed repeatedly with ethanol and methylene chloride, and dried *in vacuo*. The esterified polymer weighed 6.15 g. The increase in weight of 0.75 g indicated the leucine content of the resin to be approximately 0.47 mmol/g.

***p*-Methoxybenzyloxycarbonyl-D-phenylalanylprolylvalyl-δ-*p*-nitrobenzyloxycarbonylornithylleucyl-D-phenylalanylprolylvalyl-δ-*p*-nitrobenzyloxycarbonylornithylleucyl-Resin (1).** The above resin (5.5 g (2.6 mmol with regard to leucine)) was placed in a reaction vessel of the type described by Merrifield for a repeated deblocking, rinsing, and coupling procedure. The following cycle was used for stepwise addition of the appropriate *p*-methoxybenzyloxycarbonyl amino acids. The resin was rinsed with acetic acid (three 20-ml portions), shaken for 30 min in 1 *N* HCl in acetic acid in the presence of 2 ml of anisole (two 20-ml portions), rinsed with acetic acid (three 20-ml portions), with anhydrous ethanol (three 20-ml portions), and with dimethylformamide (three 25-ml portions), and shaken for 10 min in 10% triethylamine in dimethylformamide (two 25-ml portions) and with methylene chloride (three 25-ml portions). The resin was then suspended in 25 ml of methylene chloride containing an excess of the appropriate *p*-methoxybenzyloxycarbonyl amino acid (2.5-fold molar excess for first step increasing linearly to 3.5-fold molar excess at last step) and shaken for 15 min. Equimolar *N,N'*-dicyclohexylcarbodiimide was added in 5–10 ml of methylene chloride and shaking was continued for 4 hr. The coupling steps were terminated by rinsing with methylene chloride (three 25-ml portions) and with anhydrous ethanol (three 20-ml portions). When *p*-methoxybenzyloxycarbonylvaline was coupled to the amino end of the polymer and when the proline carboxyl was coupled to the amino group of the preceding valyl residue, the reaction time was prolonged to 8 hr because of the relatively depressed reactivity of the valyl residue due to steric hindrance of the isopropyl side chain. After completion of the last coupling cycle the *p*-methoxybenzyloxycarbonyl-decapeptidyl-resin was dried *in vacuo* (9.48 g). The weight increase was 3.98 g.

***p*-Methoxybenzyloxycarbonyl-D-phenylalanylprolylvalyl-δ-*p*-nitrobenzyloxycarbonylornithylleucyl-D-phenylalanylprolylvalyl-δ-*p*-nitrobenzyloxycarbonylornithylleucyl Hydrazide (2).** The protected peptidyl resin **1** (9.48 g) was suspended in 45 ml of dimethylformamide, 4.5 ml (92 mmol) of 100% hydrazine hydrate was added, and the mixture was allowed to stir at room temperature for 2 days. The resin was filtered and washed with three 20-ml portions of dimethylformamide. The combined filtrates were evaporated *in vacuo* to a volume of about 20 ml and a larger volume of water was added. The white crystals deposited were finely divided and filtered, washed thoroughly with water, and dried *in vacuo* over $CaCl_2$, with a yield of 4.07 g (2.52 mmol based on formula weight), mp 122–124°, $[\alpha]^{25}D$ −61° (*c* 1.11, methanol). Thin-layer chromatography showed a band at R_f 0.70 with a very faint tailing strip. Purity of the product was estimated to be 98% from the absorption measurement at 268 nm on the basis of the molar extinction coefficient of the compound of 21,300. Amino acid analysis gave the following ratio: Leu 1.00, Orn 1.00, Val 1.06, Pro 0.91, Phe 1.00. A very small amount of insoluble material remained upon acid hydrolysis.

Anal. Calcd for $C_{85}H_{114}O_{21}N_{16}\cdot 2H_2O$: C, 58.89; H, 6.81; N, 12.93. Found: C, 58.73; H, 6.90; N, 12.78.

The hydrolysate of the resin treated with dioxane-concentrated hydrochloric acid (1:1 v/v) showed no detectable amino acids upon paper chromatography.

D-Phenylalanylprolylvalyl-δ-*p*-nitrobenzyloxycarbonylornithylleucyl-D-phenylalanylprolylvalyl-δ-*p*-nitrobenzyloxycarbonylornithylleucyl Hydrazide Dihydrochloride (3). To a solution of 2.1 g (1.24 mmol) of **2** in 30 ml of ethyl acetate, 1.0 ml of anisole was added, followed by 15 ml of 3.5 *N* HCl in ethyl acetate. The solution was allowed to stand at room temperature for 1 hr and then evaporated nearly to dryness. The resulting crystalline residue was treated with ether and the slurry was filtered to yield 2.08 g of crystals (100%), mp 212–216°, which was used for the next reaction without further treatment.

***cyclo*-D-Phenylalanylprolylvalyl-δ-*p*-nitrobenzyloxycarbonylornithylleucyl-D-phenylalanylprolylvalyl-δ-*p*-nitrobenzyloxycarbonylornithylleucyl (5).** To a solution of 1.0 g (0.623 mmol) of **3** dissolved in a mixture of 11 ml of dimethylformamide and 2.7 ml of acetic acid, 1.4 ml of 1 *N* hydrochloric acid was added. The solution was cooled to −10° with a freezing mixture, 0.73 ml of 1 *N* sodium nitrite was added, and the reaction mixture was stirred at −10° for 10 min. The resulting golden yellow solution was added to 500 ml of pyridine kept at 0°. The pyridine solution was allowed to stir at 0° for 3 hr and than at 4° for 36 hr. Pyridine was removed by evaporation at 50°, and the resulting dimethylformamide solution was diluted with about 70 ml of ethyl acetate. The solution was washed successively with water, 0.5 *N* hydrochloric acid, and water, dried over Na_2SO_4, and evaporated. The residue was triturated with ether and petroleum ether (1:1 v/v) to give crystals, 0.874 g (0.583 mmol, 94%). The content of *p*-nitrobenzyloxycarbonyl was estimated to be 102% by means of the absorption measurement at 268 nm. Paper electrophoresis of a

(14) S. Sakakibara, I. Honda, M. Naruse, and M. Kanaoka, *Experientia*, **25**, 576 (1969).

hydrogenated sample of the product gave a band with the same mobility as that of natural gramicidin S but accompanied by a trace of trail and another faster moving band of *p*-toluidine.

The crude product obtained above (0.6 g) was dissolved in 50 ml of a mixture of methanol and water (8:1 v/v) and the solution was passed through a column (1.8 × 4 cm) of Dowex-50 (H^+ form) which had been equilibrated with the same solvent. The column was then washed with 50 ml of the solvent. The combined filtrates were evaporated nearly to dryness, and the residue was taken up in ethyl acetate. The solution was washed with water, dried over Na_2SO_4, and evaporated. Trituration of the residue with ether and petroleum ether (1:1 v/v) gave 0.54 g (90%) of colorless crystals; mp 228–230°; $[\alpha]^{25}D$ −149° (*c* 1.32, methanol). The content of *p*-nitrobenzyloxycarbonyl was 104% from the absorption measurement at 268 nm. The molecular weight was measured with a vapor pressure osmometer using methanol as the solvent: found, 1492; calcd for di-*p*-nitrobenzyloxycarbonyl-substituted, cyclic decapeptide, 1499.7. Amino acid analysis of the product gave the following ratio: Leu 0.98, Orn 1.00, Val 1.07, Pro 1.02, Phe 1.00.

Anal. Calcd for $C_{76}H_{102}O_{18}N_{14}\cdot 2H_2O$: C, 59.38; H, 6.90; N, 12.76. Found: C, 59.39; H, 6.98; N, 12.82.

***cyclo*-D-Phenylalanylprolylvalylornithylleucyl-D-phenylalanylprolylvalylornithylleucyl Dihydrochloride (6) (Synthetic Gramicidin S Dihydrochloride).** **5** (0.3 g (0.20 mmol)) was dissolved in 10 ml of 0.2 *N* HCl in methanol and hydrogenation was carried out for 4 hr using palladium black as a catalyst. After removal of the catalyst by filtration, the filtrate was evaporated *in vacuo*. The syrup which remained was treated with 6 ml of 1 *N* hydrochloric acid and the crystals formed were filtered, washed in portions with 6 ml of 1 *N* hydrochloric acid, and dried *in vacuo*, 0.142 g (56%). It showed a single band with mobility identical with that of natural gramicidin S upon paper electrophoresis. Recrystallization from ethanol–1 *N* hydrochloric acid gave 0.127 g (50%) of colorless crystals; mp 275–277°; $[\alpha]^{25}D$ −251° (*c* 0.530, ethanol); $[\alpha]^{25}D$ of natural gramicidin S dihydrochloride −258° (*c* 0.575, ethanol). Amino acid analysis gave the following ratio: Leu 0.96, Orn 1.00, Val 1.05, Pro 1.04, Phe 1.00.

Anal. Calcd for $C_{60}H_{92}O_{10}N_{12}\cdot 2HCl\cdot 3H_2O$: C, 56.78; H, 7.89; N, 13.25. Found: C, 57.29; H, 7.54; N, 12.56.

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Communications to the Editor

The Mechanism of Photoaquation of Hexacyanocobaltate(III)

Sir:

Several mechanistic possibilites exist for the photoaquation[1] of $Co(CN)_6^{3-}$. Adamson, Chiang, and Zinato have proposed[2] that the excited state $[Co(CN)_6^{3-}]^*$ undergoes dissociative decay to give a five-coordinate complex similar to or identical with the intermediate which has been implicated in the thermal anation of $Cc(CN)_5(H_2O)^{2-}$.[3] Another attractive pathway would involve interchange of an inner sphere CN^- ligand and a water molecule in the outer sphere of the photoexcited $Co(CN)_6^{3-}$. Radiationless decay of the electronic excited state would be concomitant with the interchange. The photointerchange mechanism is outlined in eq 1 and 2.

$$[Co(CN)_6^{3-}](H_2O)_n \xrightarrow{h\nu} [Co(CN)_6^{3-}](H_2O)_n^* \quad (1)$$

$$[Co(CN)_6^{3-}](H_2O)_n^* \xrightarrow{k_2} [Co(CN)_5(H_2O)^{2-}](CN^-)(H_2O)_{n-1} \quad (2)$$

Experimentally the two mechanisms can be distinguished since the dissociative pathway involves a five-coordinate intermediate which could be captured by nucleophiles other than water. The interchange mechanism leads only to $Co(CN)_5(H_2O)^{2-}$ unless ion pairing of an added nucleophile and $Co(CN)_6^{3-}$ occurs. The scavenging technique has been used to establish a dissociative mechanism for thermal anation of $Co(CN)_5(H_2O)^{2-}$[3] and has proved fruitful in studies of the mechanism of Hg^{2+}-catalyzed aquation of $Co(NH_3)X^{2+}$.[4]

We have studied the photoaquation of $Co(CN)_6^{3-}$ in the presence of added nucleophiles using an apparatus which allows simultaneous irradiation and sample analysis. Here we report results which suggest that interchange is the principal pathway of this reaction.

Irradiations were carried out in thermostated optical cells using a Pyrex-filtered 150-W mercury lamp source. The photolysis beam was also used for analysis by placing a Jarrell-Ash monochromator and a phototube behind the sample cell. The lamp, sample cell, and detection monochromator were mounted on an optical bench. The output from the phototube was plotted *vs.* time using a Moseley Model 7101B recorder. Care was taken to ensure that the recorder response corresponded to appropriate transmittance properties of the sample. With this irradiation and analytical tool we were able to monitor changes in the chemical content of the sample during the photochemical reaction.

Solutions containing $Co(CN)_6^{3-}$ were irradiated and the spectra showed clean initial conversion to $Co(CN)_5(H_2O)^{2-}$ as evidenced by the preservation of the isosbestic point at 330 nm.[2] Addition of nucleophilic anions, iodide or azide, in concentrations up to 1.29 *M*, did not alter the initial results although fairly rapid conversion to the iodo and the azido complexes was observed *after the accumulation of significant amounts of the aquo complex*. In other words, continuous monitoring shows that there is an induction period of many seconds in the appearance of $Co(CN)_5(X)^{3-}$ species. This is shown in Figure 1 by comparing the growth in absorption of $Co(CN)_5(H_2O)^{2-}$ at 380 nm with the

(1) Investigations of the qualitative aspects of the aqueous photochemistry of $Co(CN)_6^{3-}$ include: (a) R. Schwortz and T. K. Tede, *Chem. Ber.*, **608**, 69 (1927); (b) A. G. MacDiarmid and H. F. Hall, *J. Amer. Chem. Soc.*, **75**, 5204 (1953); 76, 4222 (1954); (c) A. W. Adamson and A. Sporer, *ibid.*, **80**, 3865 (1958); (d) L. Moggi, F. Bolletta, V. Balzani and F. Scandola, *J. Inorg. Nucl. Chem.*, **28**, 2589 (1966).

(2) A. W. Adamson, A. Chiang, and E. Zinao, *J. Amer. Chem. Soc.*, **91**, 5468 (1969).

(3) (a) A. Haim and W. K. Wilmarth, *Inorg. Chem.*, 1, 573 (1962); (b) A. Haim, R. J. Grassie, and W. K. Wilmarth, *Advan. Chem. Ser.*, **No. 49** (1965).

(4) F. A. Posey and H. Taube, *J. Amer. Chem. Soc.*, **79**, 255 (1957).