reaction which was not pretreated with anhydrous HCl, the appearance of color and of product (tlc monitoring) was not evident until 25–30 min. Repetition of this showed a color change after 22 min. After 90 min a sample (1/4) of the mixture was purified by tlc (system A), and the product was crystallized from $CH_2Cl_2$–EtOH to give 110 mg (75%) of **4** as colorless plates, mp 227–230°.

**B. $CdX_2$ Added.**—Estrone (270 mg, 1.0 mmol) was glucuronidated with chloro sugar **2** (705 mg, 2.0 mmol) using $CdCO_3$ (345 mg, 2.0 mmol) and 18 mg (0.1 mmol) of anhydrous $CdCl_2$ (Coleman and Bell Co., Norwood, Ohio) according to the general procedure B. The mixture changed color after 30 min. Separation of a sample (1/4) of the mixture by tlc (system A) and crystallization of the product from $CH_2Cl_2$–EtOH gave 91.7 mg (62.5%) of **4**, mp 226–231°.

In another experiment, estrone (250 mg, 0.925 mmol) was reacted with bromo sugar **1** according to procedure A using 410 mg (1.51 mmol) of anhydrous $CdBr_2$ (Alfa Inorganics) and 208 mg (1.50 mmol) of anhydrous $K_2CO_3$ as the catalyst–acid acceptor system. However, no product was evident by tlc (system A) and work-up of the mixture gave back 79% of the estrone and 96% of the bromo sugar.

**Registry No.**—**4**, 27537-72-0; **5**, 15087-01-1; **7**, 27610-08-8; **8**, 25591-03-1; **9**, 27610-09-9; **10**, 27537-75-3; **11**, 14982-12-8; **12**, 27537-76-4; **13**, 15087-06-6; **14**, 27570-87-2; **15**, 27610-12-4; **16**, 27537-77-5; **17**, 27537-78-6; **18**, 27537-79-7; **19**, 27537-80-0; **20**, 27537-81-1; cadmium carbonate, 513-78-0.

**Acknowledgments.**—We thank Louis M. Brancone and associates for the elemental analyses and William Fulmor and associates for the spectral data. Special thanks go to Dr. George E. Van Lear for the mass spectra and to George O. Morton for his most helpful interpretations of the nmr data.

# Synthesis of Tobacco Mosaic Virus Protein Sequence 81–85[1]

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*Received July 10, 1970*

The 81–85 segment of tobacco mosaic virus protein has been prepared by two different synthetic approaches. Synthesis of the protected pentapeptide *N*-benzyloxycarbonyl-L-threonyl-L-alanyl-L-leucyl-L-leucyl-glycine hydrazide corresponding to TMV protein 81–85 was accomplished employing as key step coupling of *N*-Z-Thr-Ala azide with Leu-Leu-Gly-OMe. The product was identical with the same pentapeptide obtained by a Merrifield solid-phase synthesis.

Synthesis of the tobacco mosaic virus protein would represent an important step toward the first total synthesis of an organism capable of replication. With this objective in view, we began a program concerned with synthesis of, at that time (1962) known, segments of the TMV protein. By 1964 the complete structure of TMV protein had been proposed with reasonable certainty.[2] Subsequently, the 120–124[3a] (solution polymer method) and 151–154[3b] (fragment condensation) units were prepared in our laboratory and units 42–46[4a] and 103–112[4b] have been prepared (solid phase technique) elsewhere. Concurrent with preparation of TMV protein fragments, we have been using certain of these peptides in an immunological[5] study of steroidal peptides[6a] and in preparation of alkaloidal peptides.[6b] The preparation reported herein of the fully protected pentapeptide *N*-Z-Thr-Ala-Leu-Leu-Gly hydrazide corresponding to TMV protein sequence 81–85 was accomplished by both conventional methods of peptide synthesis in solution and by a Merrifield solid-phase[7] synthesis.

Synthesis of pentapeptide **6** by a fragment condensation approach proceeded as follows. Condensation of *tert*-butoxycarbonyl-L-leucine with glycine methyl ester proceeded well in the presence of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDCI)[8] and gave protected dipeptide **1**. Attempts at cleaving the *tert*-butoxycarbonyl group of dipeptide **1** using trifluoroacetic acid and hydrogen chloride in methylene chloride or in methanol gave a two-component mixture. However, use of 98% formic acid[9] gave a pure product (**2**). A mixed carbonic anhydride[10] coupling procedure was used to condense *tert*-butoxycarbonyl-L-leucine with dipeptide ester **2**. By this means, the protected tripeptide **3a** was obtained in good yield. By contrast, the use of dicyclohexylcarbodiimide in methylene chloride afforded a low yield of tripeptide **3a**. The dipeptide fragment *N*-Z-Thr-Ala-OMe (**4**) was conveniently obtained as described by Hofmann, *et al.*,[11] using dicyclohexylcarbodiimide. Noteworthy at this stage of the synthesis was the observation that *N*-ethyl-5-phenylisoxazolium 3′-sulfonate (WRK)[12] in acetonitrile or nitromethane, or EDCI in methylene chloride, led to consistently low yields of the protected dipeptide **4**. Hydrazinolysis of Z-Thr-Ala-OMe **4** to

(1) Contribution XI of the series Structural Biochemistry. For part X, see P. Brown and G. R. Pettit, *Org. Mass Spectrom.*, **3**, 67 (1970). We are grateful to the National Science Foundation for financial support by Grants GB-4939 and GB-8250.

(2) G. Funatsu, A. Tsugita, and H. Fraenkel-Conrat, *Arch. Biochim. Biophys.*, **105**, 25 (1964).

(3) (a) B. Green and L. Garson, *J. Chem. Soc. C*, 401 (1969); (b) G. R. Pettit and S. K. Gupta, *J. Chem. Soc.*, 1208 (1968).

(4) (a) J. D. Young, C. Y. Leung, and W. A. Rombauts, *Biochemistry*, **7**, 2475 (1968). (b) J. M. Stewart, J. D. Young, E. Benjamini, M. Shimizu, and C. Y. Leung, *ibid.*, **5**, 3396 (1966); J. D. Young, E. Benjamini, J. M. Stewart, and C. Y. Leung, *ibid.*, **6**, 1455 (1967).

(5) For example, the 93–112 and 102–112 units of TMV protein have been reported to be antigenic areas: J. D. Young, E. Benjamini, M. Shimizu, and C. Y. Leung, *ibid.*, **5**, 1481 (1966); E. Benjamini, M. Shimizu, J. D. Young, and C. Y. Leung, *ibid.*, **8**, 2242 (1969).

(6) (a) G. R. Pettit, R. L. Smith, and H. Klinger, *J. Med. Chem.*, **10**, 145 (1967); G. R. Pettit and N. H. Rogers, *J. Org. Chem.*, manuscript in preparation. (b) G. R. Pettit and S. K. Gupta, *J. Chem. Soc. C*, 1208 (1968).

(7) R. B. Merrifield, *Advan. Enzymol. Relat. Subj. Biochem.*, **32**, 221 (1969).

(8) J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, **26**, 2525 (1961). The abbreviations for amino acids, peptides, and coupling reagents are those recommended by the IUPAC Commission on nomenclature, *Biochemistry*, **5**, 2485 (1966), and those adopted by G. R. Pettit in "Synthetic Peptides," Vol. I, Van Nostrand–Reinhold, New York, N. Y., 1970.

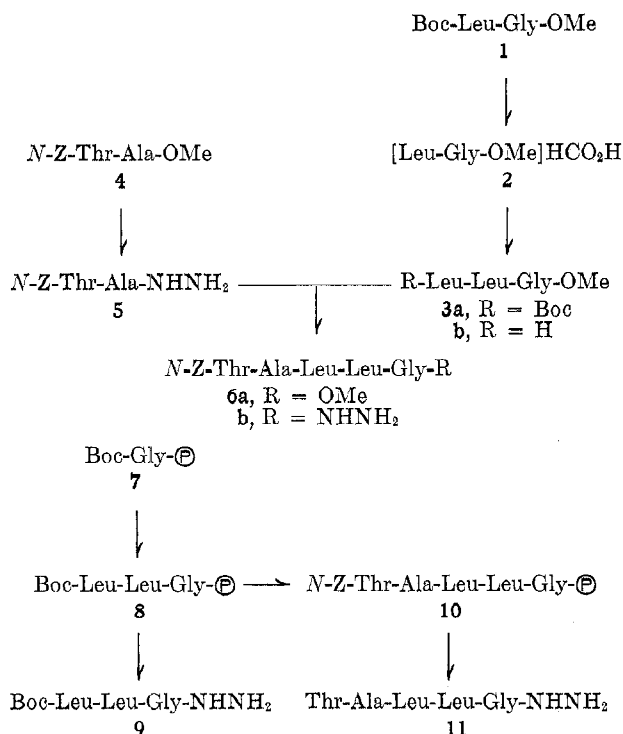
(9) B. Halpern and D. E. Nitecki, *Tetrahedron Lett.*, 3031 (1967).

(10) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, **89**, 5012 (1967).

(11) K. Hofmann, R. Schmiechen, R. D. Wells, Y. Wolman, and N. Yanaihara, *ibid.*, **87**, 611 (1965).

(12) R. B. Woodward and D. J. Woodman, *J. Org. Chem.*, **34**, 2742 (1969); R. B. Woodward, R. A. Olofson, and H. Mayer, *Tetrahedron*, **S8**, 321 (1966).

yield the corresponding hydrazide **5** was easily performed employing hydrazine in methanol.[11] Condensation of protected dipeptide **5** with tripeptide ester **3b** was achieved, albeit in low yield, by an azide procedure. The protected pentapeptide **6a** was obtained as an amorphous powder which, as evidenced by thin layer chromatography, was homogeneous. A quantitative amino acid analysis demonstrated the presence of threonine, alanine, leucine, and glycine in the molar ratios 1.0, 1.0, 2.1, and 1.1, respectively. The structure of pentapeptide **6a** was further confirmed by a diagnostic field ionization mass spectrometry study which we have already summarized.[1]




For the solid-phase synthesis of protected pentapeptide **6a**, a styrene–2% divinylbenzene copolymer was washed and chloromethylated as described by Merrifield.[7,13] The polymeric benzyl ester **7** was formed by reaction between Boc-Gly triethylammonium salt and the chloromethylated polymer. The approximate yield was determined by weight increase as suggested by Khosla.[14] The deprotection, washing, and coupling sequence with addition of each amino acid was also similar to that outlined by Khosla. Except for the last step, in which the *p*-nitrophenyl active ester technique was used,[15] the coupling method was DCCI in methylene chloride.

The growing peptide chain was analyzed at the protected tripeptide stage by subjecting an aliquot of resin to hydrazinolysis and comparison of the cleavage product with an authentic specimen of tripeptide hydrazide **9**. A several-component mixture was detected by tlc but the most prominent component had the same $R_f$ value as a specimen of hydrazide **9** prepared from protected tripeptide **3a**. Hydrazinolysis at the pentapeptide stage (**10**) afforded hydrazide **6b** in 17% overall yield based on the Boc-Gly-polymer. The amorphous product **6b** displayed a single spot on a thin layer chromatogram as did the N-deprotected derivative **11**. By thin layer chromatographic and mass spectral comparison, as well as amino acid analysis, the solid-phase product **6b** was identical with the substance obtained by the fragment condensation approach. In both syntheses the only evidence for a side product reflecting some racemization was detected during purification of protected dipeptide **4**. Preparation of pentapeptide hydrazide **6b** by the solid-phase approach proved to be most economical in terms of time and yield.

(13) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).
(14) M. C. Khosla, R. B. Smelby, and F. M. Bumpus, *ibid.*, **6**, 754 (1967).
(15) M. Bodanszky and M. A. Ondetti, *Chem. Ind.* (*London*), 26 (1966).

## Experimental Section

Alanine, leucine, and threonine were of the L configuration. The resin was styrene–2% divinylbenzene copolymer beads-X2, 200–400 mesh, lot no. 6075-31 from the Dow Chemical Co. The beads were washed thoroughly with 1 *N* sodium hydroxide, 1 *N* hydrochloric acid, water, dimethylformamide, and methanol. After drying at 95° (0.3 mm) for 48 hr, the polymer was stored in a desiccator over phosphorus pentoxide and used as required. Chloromethylation of the resin was conducted essentially as described by Merrifield.[13] The product was found to contain 5.42% chlorine.

Extracts of aqueous solutions were dried over magnesium sulfate. Thin layer chromatograms were prepared with silica gel HF-254 (E. Merck, AG, Darmstadt, Germany) unless otherwise noted. The thin layer plates were developed by ultraviolet light and/or ninhydrin. Each analytical sample was colorless and homogeneous as evidenced by tlc. Melting points were determined using a Kofler melting point apparatus and are corrected. Elemental microanalyses were provided by Dr. A. Bernhardt, Mikroanalytisches Laboratorium, 5251 Elbach uber Engelskirchen, West Germany. Proton magnetic resonance and optical rotatory dispersion measurements were conducted by Miss K. Reimer employing, respectively, an A-60 Varian spectrometer (deuteriochloroform solution with tetramethylsilane as internal standard) and a Jasco ORD-UV-5 instrument at 25° (ethanol solution). The amino acid analyses were performed by Mr. R. Storm, of our department, using a Beckman Spinco 120-C amino acid analyzer. All solvents were removed at temperatures below 25° using a rotating evaporator.

**Boc-Leu-Gly-OMe** (1).—Triethylamine (1.92 ml) was added to a mixture of Boc-L-leucine[16] monohydrate (4.2 g) and methyl glycinate hydrochloride (2.25 g) in methylene chloride (70 ml) at 0°. After 5 min 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (4.0 g) was added. The homogeneous reaction mixture was kept at 0° for 5.5 hr and then washed successively with water (two 40-ml portions), 2% sodium carbonate solution (two 30-ml portions), and water (one 20-ml portion). Removal of solvent gave a white solid which crystallized from methylene chloride–hexane as needles (3.4 g) of methyl Boc-leucyl glycinate: mp 132.5–133° [two further crystallizations from the same solvent combination raised the melting point to 132.8–133° (lit.[17] mp 128–131°)]; pmr $\delta$ 0.93 (d, $J$ = 5 Hz, isopropyl methyls), 1.45 (*tert*-butyl methyl groups), 1.6 (br hump, $(CH_3)_2CHCH_2$), 3.75 (s, $CH_3OC{=}O$), 4.05 (d, $J$ = 5.5 Hz, $NHCH_2C{=}O$), 4.3 (unresolved m, $C{=}ONHCH$); RD $[\alpha]_{589}$ −24.9°, $[\alpha]_{400}$ −66.4°, $[\alpha]_{300}$ −166° (*c* 0.723).

*Anal.* Calcd for $C_{14}H_{26}N_2O_5$: C, 55.6; H, 8.6; N, 9.27. Found: C, 55.22; H, 8.89; N, 9.3.

**Cleavage of the N-Protecting Group from Boc-Leu-Gly-OMe** (1). **A. Trifluoracetic Acid.**—The acid (3 ml) was added to peptide 1 (0.13 g) at 25°. After 25 min the TFA was removed (*in vacuo* at 25°), and the residue examined by tlc. Two components of comparable intensity ($R_f$ values 0.5 and 0.6 in 5:1:4 1-butanol–water–acetic acid) were revealed. The N-protected dipeptide had an $R_f$ value of 0.93 in the same tlc system. When the reaction was repeated with TFA for 1 min, a similar pattern on tlc was observed.

**B. By Hydrogen Chloride.**—The dipeptide 1 (80 mg) was dissolved in methylene chloride (5 ml) at 25°, and methylene chloride saturated with HCl gas at 0° was added. After 30 min

(16) E. Schnabel, *Justus Liebigs Ann. Chem.*, **702**, 188 (1967).
(17) F. C. McKay and N. F. Albertson, *J. Amer. Chem. Soc.*, **79**, 4686 (1957).

at 25° the solvent was removed. Tlc (see method A) showed a component at $R_f$ 0.5 and a less intense one at $R_f$ 0.35. The reaction was repeated as follows. Methanol saturated with hydrogen chloride gas at 10° (1 ml) was added to Boc-Leu-Gly-OMe (70 mg). Effervescence was immediate and subsided over 5 sec. After a total time elapse of 1 min the methanol was removed (*in vacuo*) and the residual yellow oil examined by tlc. One component at $R_f$ 0.5 and a trace of another at $R_f$ 0.4 was revealed.

**C. By 98% Formic Acid.**—A solution of Boc-Leu-Gly-OMe (0.60 g) in 98% formic acid (10 ml) was kept at 17° for 3 hr. Removal (*in vacuo*) of the formic acid at 23° gave a clear oil which did not solidify on trituration with dry ether. Tlc (see method A) showed one component at $R_f$ 0.5. The ether was removed at 20° and the residue of formate **2** was stored *in vacuo* over sodium hydroxide pellets for 48 hr. The formate partially solidified and was noticeably hygroscopic.

**Boc-Leu-Leu-Gly-OMe (3a).**—Boc-leucine monohydrate (1.47 g, 5.8 mmol) was dissolved in ethyl acetate (10 ml) and benzene (100 ml). The solvents were removed *in vacuo*, the vitreous residue was dissolved in dry tetrahydrofuran (100 ml) and cooled to −15°, and N-methylmorpholine (0.65 ml, 5.8 mmol) was added and followed in 4 min by isobutyl chloroformate (0.8 ml, 5.8 mmol). After an activation time of 2 min at −10°, a solution derived from methyl L-leucylglycinate formic acid salt (from 5.8 mmol of Boc-Leu-Gly-OMe) and *N*-methylmorpholine (0.65 ml) in tetrahydrofuran (20 ml at 0°) was added. The reaction mixture was stirred at −10° for 5 min and then allowed to warm to 21° during 50 min. Most of the solvent was removed and the residue partitioned between ethyl acetate (100 ml) and water (50 ml). Successive washing of the organic phase with 2% citric acid (three 15-ml portions), water (one 10-ml portion), 2% sodium carbonate (four 20-ml portions), and saturated sodium chloride (one 10-ml portion) gave, after removal of solvent, a clear oil (2.1 g) which separated from benzene–ligroin (during 18 hr) as very small needles (1.24 g, first crop). The Boc-Leu-Leu-Gly-OMe melted at 139–140°. The melting point was unchanged on recrystallization from benzene–ligroin and a tlc ($R_f$ 0.77) using 9:1 chloroform–ethanol showed only one component.

*Anal.* Calcd for $C_{20}H_{37}O_6N_3$: C, 58.74; H, 9.1; N, 9.8. Found: C, 58.86; H, 8.90; N, 10.01.

***N*-Z-Thr-Ala-OMe (4). A. DCCI Method.**—Essentially as previously reported[11] DCCI (2.2 g) was used to condense carbobenzoxy-L-threonine (2.53 g) with L-alanine methyl ester hydrochloride (1.4 g) in the presence of *N*-methylmorpholine (1.15 ml) and dry methylene chloride at 0°. The resulting white solid (3.4 g, 100% yield crude) upon tlc showed one component at an $R_f$ value of 0.7 (chloroform–ethanol 9:1) and a trace (*ca.* 1–5%) of another component at a slightly higher $R_f$ value. More product (0.35 g) was isolated from the mother liquors. Crystallization from ethyl acetate–ligroin gave needles (2.0 g, first crop) of Z-Thr-Ala-OMe: mp 128–129° [two further crystallizations from benzene–hexane raised the melting point to 130–130.5° (lit.[11] mp 127–129°)]; pmr δ 1.2 (d, $J$ = 6.5 Hz), 1.39 (d, $J$ = 7.5 Hz), 3.6 (br hump, hydroxyl), 3.75 (s, methyl ester), 5.14 (s, benzyl), 7.38 (aromatic protons); RD $[\alpha]_{589}$ −13.3°, $[\alpha]_{400}$ −31.5°, $[\alpha]_{300}$ −76.0 (*c* 0.476).

*Anal.* Calcd for $C_{16}H_{22}N_2O_6$: C, 56.80; H, 6.51; N, 8.28. Found: C, 56.77; H, 6.66; N, 8.26.

**B. EDCI Method.**—Triethylamine (0.7 ml) was added to a mixture of *N*-benzyloxycarbonyl-L-threonine (0.77 g, 3 mmol) and L-alanine methyl ester hydrochloride (0.42 g, 3 mmol) in methylene chloride (30 ml) at 0°. After 10 min 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (0.768 g, 4 mmol) was added and after 4 hr at 0° methylene chloride (20 ml). The solution was washed with water (two 20-ml portions) and 2% sodium carbonate (five 15-ml portions). Removal of the solvent gave a solid (0.80 g, 74% crude) which separated from methylene chloride–hexane as colorless blades (0.48 g) of dipeptide **4**, mp 127.5–129.2°.

**C. WRK Method.**—To *N*-ethyl-5-phenylisoxazolium 3′-sulfonate (1.01 g) in acetonitrile (18 ml) at 0° was added *N*-benzyloxycarbonyl-L-threonine (1.012 g, 4 mmol) and *N*-methylmorpholine (0.4 ml) in acetonitrile (14 ml). The cooling bath was removed and the stirred reaction mixture was allowed to warm to 25°. After 65 min the solution had clarified, and a solution of L-alanine methyl ester hydrochloride (0.56 g) in *N*-methylmorpholine (0.4 ml)–acetonitrile (12 ml) was added. Stirring was contiued at 25° for 22.5 hr. The acetonitrile was removed and replaced by ethyl acetate (100 ml), and the solution was washed with successive portions of water (one 20-ml portion), 4% sodium carbonate (two 30-ml portions), water (one 10-ml portion), 4% hydrochloric acid (two 20-ml portions), and finally with water (two 25-ml portions). Removal of solvent afforded a solid which formed hair-like crystals from methylene chloride–ligroin, 0.34 g, mp 129–129.8°. A second crop (0.14 g) had mp 124–128°.

***N*-Z-Thr-Ala-Leu-Leu-Gly-OMe (6a).**—Sodium nitrite (0.22 g) in water (1 ml) at 0° was added to a solution of hydrazide **5** (0.46 g, 1.36 mmol)[11] in cold (ice bath) dimethylformamide (20 ml) and 2 *N* hydrochloric acid (10 ml). The mixture was kept at 0–5° for 6 min and then extracted with ice-cold ethyl acetate (20 ml). The extract was washed with cold saturated sodium bicarbonate solution and with cold saturated sodium chloride solution. The ethyl acetate solution was *dried* (sodium sulfate) and added to a cold solution derived from Leu-Leu-Gly-OMe formate (from 0.58 g of protected tripeptide **3a**) and *N*-methylmorpholine (0.15 ml) in ethyl acetate (15 ml). The reaction mixture was maintained at 0° for 21 hr, and the gelatinous white precipitate which formed was dissolved by addition of ethyl acetate (50 ml). The solution was washed with successive portions of ice-cold 2% citric acid (four 15-ml portions), saturated sodium chloride solution (one 10-ml portions), 1 *N* sodium bicarbonate (two 15-ml portions), and saturated sodium chloride solution (one 10-ml portion). Removal of solvent gave a gelatinous solid which led to a powder (0.14 g, 16% yield) on storage *in vacuo*. Tlc showed essentially one component ($R_f$ 0.5, chloroform–ethanol 9:1); attempted crystallization from methanol gave a white powder, mp 227–230°. The amino acid analysis was performed as summarized below for hydrazide **6b** (obtained *via* solid-phase synthesis) and gave values of 1.0, 1.01, 2.13, and 1.07, respectively, for Thr, Ala, Leu, and Gly. The mass spectrum of pentapeptide **6a** has already been described in detail.[1]

Preparation of hydrazide **6b** was easily achieved by the method noted for obtaining dipeptide **5**. The hydrazide **6b** was *identical* (tlc, mass spectrum, and amino acid analysis) with a sample obtained by the resin method (see below).

**Boc-Gly-Polymer (7).**—Boc-glycine (2.70 g) was condensed with the chloromethylated resin (14.4 g) in absolute ethanol (30 ml) containing triethylamine (2.2 ml). The mixture was heated at reflux for 23 hr with protection from moisture. The resin was collected and washed with successive portions of ethanol (200 ml), water (200 ml), and methanol (200 ml) and dried at 25° (1 mm) for 24 hr. The dried resin weighed 15.7 g. The increase in weight represented incorporation of 7.2 mmol of Boc-glycine or 0.46 mmol of Boc-glycine per gram of *N*-Boc-Gly-resin.

**Boc-Gly-Polymer to Boc-Ala-Leu-Leu-Gly-Polymer.**—Starting with Boc-glycyl-polymer (15.1 g containing 6.9 mmol of Boc-Gly), the following series of reactions was performed. The resin was washed with acetic acid (three 75-ml portions), and the protecting group was cleaved with 1 *N* hydrochloric acid in acetic acid (75 ml) during 30 min. The resin was again washed with acetic acid (three 75-ml portions), absolute ethanol (three 75-ml portions), and dimethylformamide (three 75-ml portions). The hydrochloride salt was removed with triethylamine (7.5 ml) in dimethylformamide (over a 10–15-min period). The resin was washed with dimethylformamide (three 75-ml portions) and methylene chloride (three 75-ml portions). At this point 14 mmol of the appropriate Boc-amino acid in methylene chloride (75 ml) was added with ice cooling. After 10 min of mixing, 14 mmol of DCCI in methylene chloride (20 ml) was added, and the mixture shaken for 2 hr with ice cooling and overnight at ambient temperatures. Next, the resin was washed with methylene chloride (three 75-ml portions) and ethanol (three 75-ml portions). The same cycle was repeated for each addition of an amino acid unit to the growing peptide chain on the resin except that with Boc-Ala no ice cooling was used in the coupling reaction. Also, 10 min after the first addition of DCCI the resin had turned bright yellow and remained highly colored (either yellow or brown) thereafter.

Analysis of the peptide-resin after the addition of the third amino acid unit was accomplished as follows. The dried resin (80 mg) was treated with anhydrous hydrazine (0.4 ml) in absolute ethanol (3 ml) for 48 hr at 25°. The solution was filtered, the filtrate evaporated, and the residue stored *in vacuo* over phosphorus pentoxide for 12 hr. A silica gel G tlc in the system 1-butanol–acetic acid–water (5:1:4) and vizualization by brief exposure to hydrogen chloride vapor followed by ninhydrin spray showed that the main component, an orange spot, had the

same $R_f$ value as the Boc-Leu-Leu-Gly hydrazide, prepared by alternate synthesis (*vide infra*).

**Boc-Leu-Leu-Gly Hydrazide** (9).—To Boc-Leu-Leu-Gly-OMe (70 mg) in methanol (1 ml) was added hydrazine hydrate (4 drops). After 24 hr at 25° the solvent was removed giving a clear oil which showed one component on tlc (silica gel G, 1-butanol–acetic acid–water, 5:1:4, $R_f$ 0.6).

***N*-Z-Thr-Ala-Leu-Leu-Gly-Polymer** (10).—To a solution of *N*-carbobenzoxy-L-threonine (3.2 g, 12 mmol) in cold (ice bath) dry ethyl acetate (30 ml) was added *p*-nitrophenol (1.83 g, 13.2 mmol). The cold solution was stirred 5 min and dicyclohexylcarbodiimide (2.72 g, 13.2 mmol) in dry ethyl acetate (10 ml) was added. Stirring was continued 65 min at ice-bath temperature. After removing the ice bath, for 15 min glacial acetic acid (2 drops) was added. The precipitated dicyclohexylurea was collected and washed with ethyl acetate (10 ml). Solvent was removed giving a yellow oil which did not crystallize. The oil, which showed predominently one component on tlc (silica gel G, chloroform–ethanol, 18:1, $R_f$ 0.5), was used in the peptide-forming reaction without further purification. Coupling to the tetrapeptide-polymer was performed using the Z-Thr-ONp (14 mmol) in DMF (50 ml). The reaction was allowed to proceed 17 hr at 25°. At that point the resin was collected and washed with dimethylformamide (five 80-ml portions) and ethanol (three 75-ml portions).

***N*-Z-Thr-Ala-Leu-Leu-Gly Hydrazide** (6b).—The pentapeptide-polymer (10) was treated with dimethylformamide (50 ml) for 60 min. Anhydrous hydrazine (14 ml) was added and agitation continued 67 hr. The resin was collected and washed with dimethylformamide (two 50-ml portions). The combined filtrate and washings were evaporated at 45° *in vacuo* to a yellow residue which was triturated with water (30 ml). Precipitation of the solid from ethanol gave an amorphous powder (0.70 g), 17% yield based on Boc-Gly-polymer which showed one spot on tlc (silica gel G, 1-butanol–acetic acid–water 5:1:4) with $R_f$ value identical with that of *N*-Z-Thr-Ala-Leu-Leu-Gly hydrazide obtained from methyl ester **6a**.

The residual polymer was treated with anhydrous hydrazine (50 ml) for 48 hr. Evaporation of the filtrate after addition of water did not leave a residue. Hydrazinolysis of the pentapeptide-resin was therefore complete after the first treatment with hydrazine.

Hydrazide **6b** (7.63 mg) was treated with 2 *N* hydrogen bromide–acetic acid (10 ml), in which it slowly dissolved. After 110 min the solvent was removed at 40° *in vacuo* after water (1 ml) added to the residue. Tlc (silica gel G, 1-butanol–acetic acid–water 5:1:4) and vizualization with ninhydrin showed one pink spot at $R_f$ 0.41. Concentrated hydrochloric acid (5 ml) and water (4 ml) were added to the solution which was then heated at reflux for 21 hr. The water was removed at 50° *in vacuo* and the residue dissolved in citrate buffer ("sample diluter" 100 ml). A 1-ml aliquot was used in the amino acid analysis which showed the presence of threonone, alanine, leucine, and glycine only, in the molar ratio 1:1.09:2.19:1.03, respectively.

**Registry No.**—**1**, 27610-07-7; **3a**, 27545-11-5; **4**, 2483-53-6; **6a**, 27545-13-7; **6b**, 27545-14-8; **7**, 27536-85-2.

# The Structure of Viomycidine

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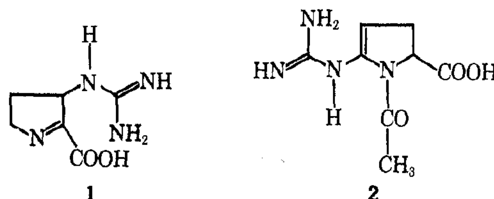
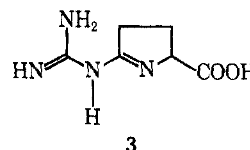
*Received September 17, 1970*

Viomycidine, a guanidino amino acid obtained from the antibiotic vioymcin by acid hydrolysis, has been shown to be 7-*endo*-carboxy-3-imino-2,4,6-triazabicyclo[3.2.1]octane. The structural assignment was made primarily on the basis of nuclear magnetic resonance evidence and oxidation of viomycidine to 3-guanidinopyrrole and of viomycidine methyl ester to 2-carbomethoxy-3-guanidinopyrrole. Earlier degradative evidence is discussed in terms of the new structure.

Viomycin, a polypeptide produced by *Streptomyces puniceous* and *Streptomyces floridae*,[1] shows marked tuberculostatic activity[2,3] but because of its toxicity has remained a secondary drug in the chemotherapy of tuberculosis.[4] Structural work on viomycin is being pursued in several laboratories and should be completed in the near future. Vigorous acid hydrolysis of viomycin gave some known amino acids and a new one which has been named viomycidine.[5–7] This fragment is optically active, has $pK_a$ values of 1.3 (estimated), 5.50, and 12.6 (in water) and a composition of $C_6H_{10}O_2N_4$, and forms well-defined salts. Oxidation with nitric acid or with permanganate gave guanidine **15**, while alkaline hydrolysis led to pyrrole-2-carboxylic acid (**14**), 2-aminopyrimidine (**16**), and glycine (**17**). Viomycidine was reported to be susceptible to catalytic hydrogenation[6,7] and this finding led to the suggestion that the molecule contains a second carbon–nitrogen double bond in addition to the nonreducible double bond of the guanido group. Based on these findings and some physical properties structure **1** was proposed for viomycidine.[6,8] Later on, in an experiment designed to serve the twofold purpose of locating the




double bond in the ring and the point of attachment of the guanido group, acetylviomycidine was ozonized.

(1) V. Ševčik, "Antibiotica aus Actinomyceten," Gustav Fischer Verlag, Jena, 1963.

(2) A. C. Finlay, G. L. Hobby, F. Hochstein, T. M. Lees, F. F. Lenert, J. A. Means, S. Y. P'an, P. P. Regna, J. B. Rontien, B. A. Sobin, K. B. Tate, and J. H. Kane, *Amer. Rev. Tuberc.*, **63**, 1 (1951).

(3) Q. R. Bartz, J. Ehrlich, J. D. Mold, M. A. Penner, and R. M. Smith, *ibid.*, **63**, 4 (1951).

(4) R. S. Mitchell and J. C. Bell, "Modern Chemotherapy of Tuberculosis," Medical Encyclopedia, Inc., New York, N. Y., 1958.

(5) T. H. Haskell, S. A. Fusari, R. P. Frohardt, and Q. R. Bartz, *J. Amer. Chem. Soc.*, **74**, 599 (1952).

(6) J. H. Bowie, A. W. Johnson, and G. Thomas, *Tetrahedron Lett.*, 863 (1964).

(7) J. R. Dyer, H. B. Hayes, E. G. Miller, Jr., and R. F. Nassar, *J. Amer. Chem. Soc.*, **86**, 5363 (1964).

(8) J. R. Dyer, H. B. Hayes, and E. G. Miller, Jr., 3rd Interscience Conference for Antimicrobial Agents and Chemotherapy, Washington, D. C., Oct 1963; *cf.* D. Perlman, *Nature*, **201**, 456 (1964).