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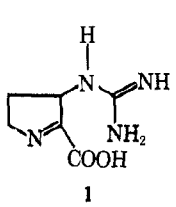
Viomycidine, a guanidino amino acid obtained from the antibiotic vioymcin by acid hydrolysis, has been shown to be 7-*endo*-carboxy-3-imino-2,4,6-triazabicyclo[3.2.1]octane. The structural assignment was made primarily on the basis of nuclear magnetic resonance evidence and oxidation of viomycidine to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



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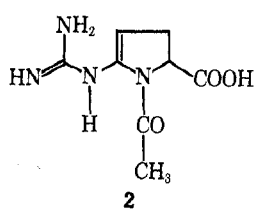

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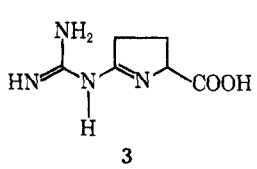

3

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).

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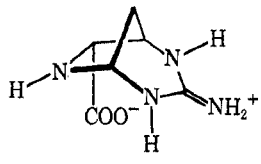
(7) J. R. Dyer, H. B. Hayes, E. G. Miller, Jr., and R. F. Nassar, *J. Amer. Chem. Soc.*, **86**, 5363 (1964).

(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).

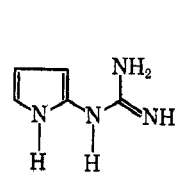
Guanidine and racemic aspartic acid were the only observable products suggesting that acetylviomycidine and viomycidine were represented by structures **2** and **3**, respectively.[7]

We had reservations concerning the stabilities of compounds such as **1** and **3** to vigorous acid hydrolysis and in this paper describe evidence in favor of structure **4** for viomycidine.



4

We began our own thinking on the structure of viomycidine by accepting the presence of a 2-carboxypyrrolidine moiety, and new evidence in favor of this postulate was provided by the mass spectrum which agreed with that of pyrrole-2-carboxylic acid with an additional peak at *m/e* 59 corresponding to the molecular ion of guanidine. On the other hand the existing evidence was not sufficient to permit placing of the guanidine group with any degree of confidence. Our hope that dehydrogenation of viomycidine would lead to a pyrrole whose substitution pattern could be ascertained by nuclear magnetic resonance spectroscopy was confirmed by experiment. Oxidation with mercuric acetate in aqueous acetic acid gave a crystalline acetate $C_7H_{12}N_4O_2$. A violet color with Ehrlich reagent and a positive Sakaguchi[9,10] test indicated the presence of a pyrrole and a monosubstituted guanidine, respectively. That the oxidation product lacked the original carboxyl group of viomycidine was evident from the ultraviolet spectrum which displayed end absorption only while pyrrole-2-carboxylic acid has a maximum at 258 nm (ϵ 12,600).[11] This was confirmed by a nuclear magnetic resonance spectrum which in D_2O displayed in addition to the three-proton singlet due to the acetate ion three aromatic protons at δ 6.12 (1 H, t, J = 2 Hz) and 6.82 (2 H, d, J = 2 Hz). Although the nmr spectra of neither amino- nor guanidinopyrroles seem to be recorded in the literature, we tentatively concluded from the absence of coupling constants larger than 3 Hz[12-14] and the presence of two low field aromatic protons that the oxidation product was 3-guanidinopyrrole acetate (**6**). This was confirmed by synthesis. Catalytic reduction of 2- and 3-nitropyrrole[15] separately over a platinum catalyst in ethanol containing 1 equiv of sodium ethoxide (required to suppress decomposition of the reduction products[15]) gave the exceptionally unstable 2- and 3-aminopyrroles, respectively. Immediate condensation with *S*-methylisothiuronium sulfate[16] produced the corresponding guanidinopyrroles **5** and **6** in low yield. Preliminary comparison by chromatographic techniques and Ehrlich color tests led to the conclusion that the oxidation product of viomycidine is 3-guanidinopyrrole (**6**).



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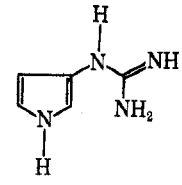
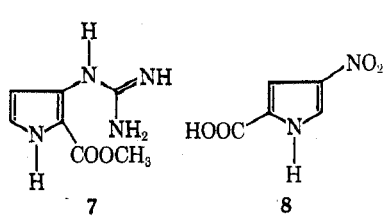


6

On the basis of the preceding discussion the carboxyl group in viomycidine must be attached to either C_2 or C_5 of the pyrrolidine ring. To differentiate between these alternatives, viomycidine had to be transformed to a pyrrole retaining both guanido and carboxy groups. Oxidation of viomycidine methyl ester dihydrochloride with mercuric acetate resulted in the pyrrole **7** with ultraviolet absorption at 266 nm (ϵ 13,500). The nuclear magnetic resonance spectrum in D_2O confirmed the presence of a methyl ester and the appearance of one-proton signals at δ 6.38 (d, J = 3 Hz) and 7.17 (d, J = 3 Hz) favored a 2,3-disubstituted pyrrole because proton coupling between C_2 and C_4 positions in pyrroles is smaller than 2 Hz. To verify this, 2-carboxy-4-nitropyrrole (**8**)[13,17] was hydrogenated and the resulting 2-carboxy-4-aminopyrrole which was much more stable than 3-aminopyrrole condensed with *S*-methylisothiuronium sulfate. 2-Carboxy-4-guanidinopyrrole (**9**) when heated in acetic acid gave 3-guanidinopyrrole (**6**) identified beyond doubt with material prepared by oxidation of viomycidine. Methylation of the acid **9** gave the methyl ester **10** different from its isomer **7** from viomycidine. In agreement with anticipation the two aromatic protons at δ 6.87 and 7.12 in the nmr spectrum of **10** are split by only 1.8 Hz. Furthermore, the difference in chemical shift between α and β pyrrole protons (0.3 ppm) is much smaller than in **7** (0.8 ppm) and in pyrrole (0.65 ppm) due to deshielding of the β proton by the carbomethoxy group.[12,13]



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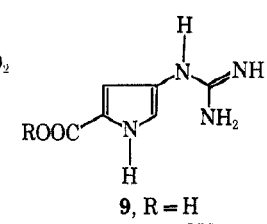

9, R = H
10, R = CH_3

With the ambiguity concerning the position of the guanido group removed, there seemed little question that viomycidine contains a 2-carboxy-3-guanidinopyrrolidine part structure. The nuclear magnetic resonance spectrum of viomycidine contains five nonexchangeable protons, and if the substance is indeed a pyrroline it should have structure **1** or **12**. The absence of an absorption pattern expected from vicinal methylene protons eliminated the former and lack of resonances at approximately δ 7 caused by a proton attached to

(9) S. Sakaguchi, *J. Biochem. (Tokyo)*, **5**, 13, 25 (1925).
(10) J. D. Mold, J. M. Ladino, and E. J. Schantz, *J. Amer. Chem. Soc.*, **75**, 6321 (1953); A. Heesing and K. Hoppe, *Chem. Ber.*, **100**, 3649 (1967).
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(13) K. J. Morgan and D. P. Morrey, *Tetrahedron*, **22**, 57 (1966).
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(15) H. Fischer and F. Rothweiler, *Ber.*, **56**, 512 (1923).
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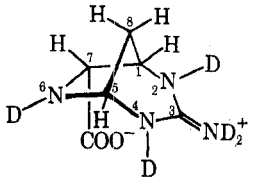
the imino group[18] removed the latter from further consideration. The bridged structure **4** on the other hand accommodates the nuclear magnetic resonance spectrum (in D_2O) with ease: C_8, δ 2.18, AB of ABXY pattern; C_7, 3.86, d, J = 4 Hz; C_1, 4.18, m; C_5,



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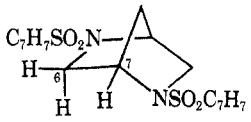
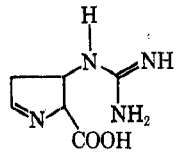


11

4.73, d of d. Comparison of J_{17} coupling (4 Hz) with those of exo C_6–C_7 (2.2 Hz) and endo C_6–C_7 (0 Hz) in **11** indicates endo configuration of the carboxy group in viomycidine.[19] The bicyclic aminoacetal structure **4** is entirely consistent with the degradation products resulting from treatment of viomycidine with base. Opening of the pyrrolidine ring leads to the dihydropyrimidine **13** which should not need much provocation to fragment into 2-aminopyrimidine (**16**) and glycine (**17**). Cleavage of the six-membered ring leads to the imine **12** which within the corresponding enamine can eject guanidine (**15**) to give pyrrole-2-carboxylic acid (**14**).



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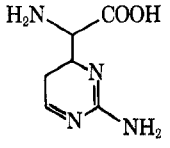

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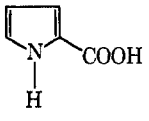

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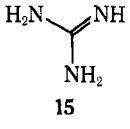

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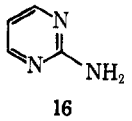

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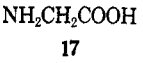
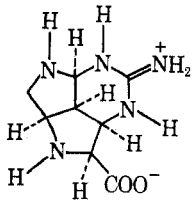


17

Structure **4** also accounts for the observed pK_a values of viomycidine. The highest value is characteristic of the guanidino group which when protonated is expected to have a drastic base weakening effect on the secondary amine function. (For example the pK_a values for ethylenediamine are 9.89 and 6.97.[20]) The lowest pK_a value is assigned to the carboxyl group of viomycidine. Oxidation of viomycidine (**4**) to pyrroles proceed *via* the pyrroline **12**, the result of an acid-catalyzed ring opening. Since the new formulation no longer contains a double bond, we reinvestigated the catalytic reduction of viomycidine. Hydrogenation over platinum in acetic acid[7] or palladium in an unspecified solvent[6] reportedly proceeds with uptake of 1 equiv of hydrogen, but no products were described and the time required for complete hydrogenation was not reported. In our hands hydrogenation of viomycidine over platinum in 0.5 *N* hydrochloric acid and over W-7 Raney nickel in water were exceedingly slow and gave two ninhydrin-positive products which were not characterized. This finding is not in disagreement with structure **4** but the formation of aspartic acid by ozonization of acetylviomycidine remains an enigma.[7]

After this investigation was completed[21] and the results quoted,[22] the structure of viomycidine was confirmed by a single-crystal X-ray structure determination of the corresponding hydrobromide.[23] The absolute configuration was not determined but it follows as shown in **4** with a high degree of certainty from that of viocidic acid **18**,[22,24] another degradation product of viomycin.



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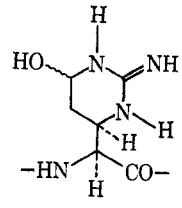

19

Both viomycidine (**4**) and viocidic acid (**18**) seem to be artifacts produced from the unit **19**[22,25] present in the intact antibiotic viomycin. Viomycidine (**4**) belongs to a family of guanidino acids discovered in antibiotics, comprising roseonine[26] (from *Streptomyces roseochromogenes*), blasticidic acid[27] (from *Streptomyces griseochromogenes*), and capreomycidine[22,28,29] (from *Streptomyces capreolus*).

Experimental Section

General.—Melting points were determined on a hot-stage microscope and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 237 grating instrument and peak intensities are given as very strong (vs), strong (s), medium (m), or weak (w). Ultraviolet spectra were recorded on a Cary Model 14 recording spectrophotometer. Nuclear magnetic resonance spectra were measured on a Varian A-60 spectrometer. The standards used were tetramethylsilane (TMS) and sodium 3-(trimethylsilyl)-1-propanesulfonate (TSPS). Thin layer chromatography (tlc) was used extensively with Merck silica gel G and aluminum oxide G, and MN 300 G cellulose powder serving as adsorbents and 1-propanol–acetic acid–water 44:12:44 (solvent A), methanol–concentrated ammonium hydroxide–water 32:1:8 (solvent B), and 1-butanol–acetic acid–water 73:10:17 (solvent C) serving as the main solvent combinations. Ninhydrin and Ehrlich spray reagents and iodine vapor were used separately and in concert to develop the plates. High voltage electrophoresis was a useful analytical tool with Whatman No. 3 MM filter paper serving as the support and acetic acid–formic acid–water 20:2:78 (pH 1.81) serving as the sole electrolyte. Typically, a potential of 35 V/cm was applied resulting in a current of 50 mA, the paper being immersed in a water-cooled bath of varsol. After 2 hr the chromatogram was retrieved, dried, and soaked by immersion with a 0.1% solution of ninhydrin in 1-butanol. Air-drying revealed most of the hydrolysate com-

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(19) P. S. Portoghese and A. A. Mikhail, *J. Org. Chem.*, **31**, 1059 (1966).
(20) M. Kotake, "Constants of Organic Compounds," Asakura Publication Co., Tokyo, 1963.
(21) J. A. Raleigh, Ph.D. Dissertation, Massachusetts Institute of Technology, Oct 1966.
(22) B. W. Bycroft, D. Cameron, L. R. Croft, A. W. Johnson, T. Webb, and P. Coggon, *Tetrahedron Lett.*, 2925 (1968).
(23) J. C. Floyd, J. A. Bertrand, and J. R. Dyer, *Chem. Commun.*, 998 (1968).
(24) P. Coggon, *J. Chem. Soc. B*, 838 (1970).
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(27) N. Otake, S. Takeuchi, T. Endo, and H. Yonehara, *Tetrahedron Lett.*, 1411 (1965).
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ponents from viomycin while heating briefly at 110° was required to develop the spot corresponding to viomycidine; urea was detected by Ehrlich spray reagent (a bright yellow color).

Viomycidine. Isolation and Characterization.—In a typical procedure viomycin sulfate (15.7 g) was hydrolyzed in 600 ml of 6 *N* hydrochloric acid at 100° (steam bath) for 6 hr (complete hydrolysis of the antibiotic was observed in this time). The deep red hydrolysate was diluted in half with distilled water and taken to dryness repeatedly *in vacuo* (at 50–60° on rotary evaporator). The last traces of hydrochloric acid were removed by passage of the hydrolysate through an Amberlite IR 4B (OH^-) column (2 × 25 cm). The column was washed with 100 ml of distilled water (effluent becomes neutral, subsequent elution of the column with dilute aqueous acid and base produced no material), the wash combined with the rest of the neutralized hydrolysate, and the combination (300 ml, pH 8) passed onto an Amberlite IRA 400 (OH^-) column (3 × 45 cm, conditioned in the usual manner). The column was eluted with distilled water and the first 500 ml of effluent combined (electrophoresis showed this fraction to be a mixture of urea and viomycidine contaminated with other components of the hydrolysate), concentrated *in vacuo* to 300 ml, and rechromatographed on an Amberlite IRA 400 (OH^-) column (3 × 45 cm). The first 450 ml of effluent from the second chromatography was largely a mixture of urea and viomycidine, the next 300 ml contained slightly impure viomycidine, and the last 600 ml of effluent (effluent was collected until it no longer gave a positive Sakaguchi reaction) contained an almost equal mixture of viomycidine and a closely related substance. The first and third fractions were combined and rechromatographed. The second fraction was taken to dryness *in vacuo* to give a crystalline residue which was subjected to fractional precipitation from an ethanol–water pair. The forecrops (132 mg) were set aside for further purification and the mother liquor was taken to dryness *in vacuo* to yield 1.48 g of purified viomycidine, mp 178–180° dec. Tlc on silica gel G (solvent A) showed this material to be of high purity with only traces of slower moving materials being present. Repeated recrystallization from a methanol–ethyl acetate solvent pair gave pure viomycidine: mp 181–182° dec; homogeneous by tlc on silica gel G (solvent A and solvent B) and by electrophoresis; $[\alpha]^{32.2}D$ −151° (*c* 1.25 in H_2O), $[\alpha]^{32.2}D$ −38° (*c* ~0.8 in aqueous HCl); $pK_a' < 2.2$, = 5.2, > 12.4 (50% aqueous EtOH); ir (KBr) 1705 (shoulder), 1670 (vs), 1608 (vs), 1600 (vs), 1575 cm^{-1} (shoulder); uv (H_2O) end absorption only; nmr (D_2O, TSPS internal standard) δ 2.18 (2 H, approximates AB of ABXY pattern), 3.86 (1 H, d, J = 4 Hz), 4.18 (1 H, m), 4.73 (1 H, d of d).

Viomycidine Monohydrochloride.—Viomycidine (337 mg) in 2 ml of water (pH 8–9 pHydrion paper) was neutralized (dilute hydrochloric acid) and the solution taken to dryness *in vacuo:* yield of monohydrochloride 290 mg; mp 200–205° dec (lit.[7] 200–208° dec); ir (KBr) 3500, 3240, 2940, 1700 (vs), 1655 (s), 1640 (shoulder), 1585 cm^{-1}; uv (H_2O) end absorption.

Viomycidine Methyl Ester Dihydrochloride.—Viomycidine monohydrochloride (290 mg) was added to 1.0 ml of thionyl chloride which had been dissolved in 5.0 ml of absolute methanol at −10°. After standing overnight at room temperature in a sealed flask, the reaction solution was taken to dryness *in vacuo*. Tlc (aluminum oxide G, methanol) showed incomplete conversion of the starting material, and the reaction mixture was treated with a further 0.25 ml of thionyl chloride in 5.0 ml of methanol as above. The methyl ester dihydrochloride was precipitated directly from the reaction mixture by the addition of ethyl ether: yield 200 mg; mp 195–200° dec; ir (KBr) 3250, 3100, 1760, 1670, 1630, 1585 cm^{-1}; uv (H_2O) end absorption only; nmr (D_2O, TSPS internal standard) δ 2.57 (2 H, t, J = 2.3 Hz), 4.02 (3 H, s), 5.77 (2 H, t, J = 3.1 Hz), the remaining CH was under the HOD peak; nmr (DMSO-d_6, TMS internal standard) δ 2.28 (2 H, s), 3.82 (3 H, s), 4.61 (2 H, t, J = 2.5 and 4.5 Hz), 5.33 (1 H, s broad), 8.04–9.29 (6 H, exchangeable NH protons). Upon hydrolysis (12 *N* hydrochloric acid, 100°, 10 hr) the methyl ester gave viomycidine as the only observable product (electrophoresis, tlc). The methyl ester was purified for analysis by recrystallization from methanol–ethyl ether.

Anal. Calcd for $C_7H_{14}N_4O_2Cl_2$: C, 32.68; H, 5.45; N, 21.79. Found: C, 32.40; H, 5.77; N, 22.02.

Viomycidine Dihydrochloride.—A solution of viomycidine (120 mg) in 5 ml of 3 *N* hydrochloric acid was taken to dryness repeatedly *in vacuo* (40°) until excess hydrochloric acid had been removed. After storage over $KOH/CaCl_2$ overnight, the crude dihydrochloride was recrystallized from methanol–ethyl ether: first crop 40 mg, mp 210–220° dec, sinter 90–100°; second crop 30 mg, mp 190–210° dec, sinter 157–160°; third crop 34 mg, white crystalline feathers, mp 190–195° dec, sinter 159–160°, and ir (KBr) 3250, 3075, 1745, 1670, 1625, 1575 cm^{-1}.

Anal. Calcd for $C_6H_{12}N_4O_2Cl_2 \cdot CH_3OH$: C, 30.56; H, 5.86; N, 20.37. Found: C, 30.38; H, 5.82; N, 20.85.

Mercuric Acetate Oxidation of Viomycidine to 3-Guanidinopyrrole Acetate (6).—Viomycidine (170 mg, 0.0010 mol) and mercuric acetate (350 mg, 0.0011 mol) were dissolved in 5% aqueous acetic acid (15 ml) and the solution was heated at 100° (oil bath) for 3 hr with stirring. The precipitated mercurous acetate was filtered off and the filtrate (pH 7) saturated with hydrogen sulfide. The precipitated mercuric sulfide was filtered off and the filtrate once again saturated with hydrogen sulfide. After filtration of the mercuric sulfide, the filtrate was taken to dryness *in vacuo* (50°, rotary evaporator) to give a partially crystalline residue which was purified by chromatography on cellulose powder. Standard grade Whatman cellulose powder (20 g) was slurried with solvent C and made into a column (2 × 20 cm) which was then washed with 150 ml of the slurry solvent. The sample (dissolved in a small amount of water) was introduced to the top of the column. Fractions (10–15 ml) were collected with the major product coming off the column after the passage of 150 ml of eluent. Evaporation of the fractions containing the major product gave 3-guanidinopyrrole acetate (6) which was homogeneous according to tlc (cellulose powder, solvent C): yield 51 mg (28%); mp 165–175° dec; recrystallized from methanol–ethyl ether, mp 168–175° dec; ir (KBr) 3450, 1680, 1640, 1535 cm^{-1}; uv (H_2O) end absorption only; nmr (D_2O, TSPS external standard) δ 2.05 (3 H, s, $OCOCH_3$), 6.12 (1 H, t, J = 2 Hz), 6.82 (2 H, d, J = 2 Hz). The compound gave a violet color with Ehrlich reagent, a purple color with a pine splint saturated with hydrochloric acid vapors, and a green color with the Sakaguchi reagent.

Anal. Calcd for $C_7H_{12}N_4O_2$: C, 45.64; H, 6.57; N, 30.42. Found: C, 45.56; H, 6.76; N, 30.22.

Mercuric Acetate Oxidation of Viomycidine Methyl Ester Dihydrochloride to 2-Carbomethoxy-3-guanidinopyrrole Hydrochloride (7).—Viomycidine methyl ester dihydrochloride (300 mg, 0.0012 mol) and mercuric acetate (440 mg, 0.0014 mol) were dissolved in 5% aqueous acetic acid (25 ml), and the solution was stirred at 75–80° for 1 hr (within 25 min ultraviolet absorption at 267 nm appeared to reach maximum intensity). The precipitated mercurous acetate was filtered off and the filtrate treated with hydrogen sulfide. Mercuric sulfide was filtered off, the filtrate treated with hydrogen sulfide, and the mercuric sulfide once again filtered. The filtrate and 5% aqueous acetic acid washings of the precipitated mercuric sulfide were combined and taken to dryness *in vacuo* to give a solid residue, yield 255 mg. The crude product was triturated with absolute ethanol (three 5-ml portions), the filtered ethanolic triturate taken to dryness *in vacuo*, and the solid residue dried over $KOH/CaCl_2$ for 12 hr. This partially purified material was chromatographed on cellulose powder (30 g of Whatman standard grade cellulose powder in ethanol–chloroform 1:1 slurry made up into a column 2 × 27 cm) after being introduced to the top of the column adsorbed on cellulose powder. Fractions containing the chromophore (267 nm, eluent ethanol–chloroform 1:1) were collected, combined, and evaporated to dryness *in vacuo*, yield 45 mg (18%). This material was slightly contaminated with components of low R_f on tlc. The product was recrystallized from ethanol–carbon tetrachloride: mp 195–198° dec (crystalline residue mp >300°); ir (KBr) 1690, 1665, 1650, 1600 cm^{-1}; uv (EtOH) 266 nm (ε 13,500); uv (H_2O) 267.5 nm; nmr (D_2O, TSPS external standard) δ 3.92 (3 H, s), 6.38 (1 H, d, J = 3 Hz), 7.17 (1 H, d, J = 3 Hz).

2-Carboxy-4-guanidinopyrrole Sulfate (9).—To 2 *N* sodium hydroxide (2.25 ml, 0.0045 mol) in distilled water (8 ml) containing platinum oxide (237 mg) was added 2-carboxy-4-nitropyrrole[13] (702 mg, 0.0045 mol) and hydrogenation commenced with vigorous stirring. Within 2 hr the hydrogenation was complete (98% of the theoretical uptake) and the pale yellow solution was decanted from the catalyst in a closed system onto powdered *S*-methylisothiouronium sulfate (417 mg, 0.030 equiv) mixed with a pinch of sodium metabisulfite and contained in a nitrogen-flushed flask. The solution was stirred at 95° (oil bath) under a stream of nitrogen for 7 hr. The dark solution was filtered and neutralized with 5 *N* sulfuric acid and a copious, crystalline precipitate formed. The reaction mixture was chilled

(0°) for 48 hr and the olive green crystals were collected by filtration, washed with a little ice–water, and air-dried, yielding 504 mg (77%): mp 203–205° dec; faint yellow Ehrlich test; insoluble in water and common organic solvents; ir (KBr) 1710, 1675 (vs), 1625 cm^{-1} (s); nmr (D_2O/NaOD, TSPS external standard) δ 6.43 (1 H, d, J = 1.6 Hz), 6.72 (1 H, d, J = 1.6 Hz). Because of its insolubility this material was used without further purification.

3-Guanidinopyrrole Acetate (6).—To anhydrous barium acetate (127.8 mg, 0.50 mmol) in 5% aqueous acetic acid (15 ml) was added 2-carboxy-4-guanidinopyrrole sulfate (9) (217 mg, 0.50 mmol) followed by mercuric acetate (175 mg, 0.55 mmol). The heterogeneous mixture was heated at 93° (oil bath) with stirring for 3 hr (the reaction mixture darkened quickly and within 15 min gave a violet color with Ehrlich reagent). The reaction mixture was cooled and filtered and the filtrate treated with hydrogen sulfide. The small amount of mercuric sulfide precipitate was filtered off and the filtrate taken to dryness *in vacuo*. Crystallization and recrystallization from methanol–ethyl ether gave 119 mg of crystalline 3-guanidinopyrrole acetate: mp 170–178° dec; ir (KBr) 1680, 1640, 1535 cm^{-1}. The synthetic product was homogeneous and identical with material obtained by oxidation of viomycidine (tlc on cellulose powder, solvent C, Ehrlich reagent, and ir spectrum).

Anal. Calcd for $C_7H_{12}N_4O_2$: C, 45.64; H, 6.57. Found: C, 45.69; H, 6.64.

2-Carboxy-4-guanidinopyrrole Hydrochloride (9).—A saturated barium hydroxide solution was added with vigorous mixing to 2-carboxy-4-guanidinopyrrole sulfate (245 mg) suspended in 10 ml of distilled water until a pH of 8–9 (Hydrion pH paper) was attained. The precipitated barium sulfate was centrifuged down and the supernatant liquid drawn off. The barium sulfate was washed with a few milliliters of distilled water and the wash combined with the supernatant liquid; the combination was acidified (carefully with dilute hydrochloric acid) and taken to dryness *in vacuo*. The residue was extracted with boiling methanol (three 3-ml portions), the extract taken to dryness *in vacuo*, and the residue recrystallized from methanol–ethyl ether. The first recrystallization gave 65 mg of impure hydrochloride. Further recrystallization from methanol–ethyl ether gave 40 mg of pure 2-carboxy-4-guanidinopyrrole hydrochloride as granular crystals: mp 179–180° dec; homogeneous upon tlc (cellulose powder, solvent C); extremely weak Ehrlich test (yellow color); ir (KBr) 3460, 3325, 3165, 1690, 1670, 1604 cm^{-1}; nmr (D_2O, TSPS external standard) δ 6.61 (1 H, d, J = 1.7 Hz), 6.89 (1 H, d, J = 1.7 Hz).

2-Carbomethoxy-4-guanidinopyrrole Hydrochloride (10).—A solution of 2-carboxy-4-guanidinopyrrole hydrochloride (9) (40 mg) in 5 ml of absolute methanol was saturated with hydrogen chloride gas. After 12 hr at room temperature in a sealed flask the methanolic solution was taken to dryness *in vacuo*, and the residual hydrogen chloride removed by repeated evaporations with methanol. The final residue was recrystallized from methanol–ethyl ether to give 24 mg of hydroscopic granular crystals: mp 103–107°; homogeneous by tlc (silica gel G, solvent A); ir (KBr) 1700, 1675, 1635, 1600, 1510 cm^{-1}; nmr (D_2O, TSPS external standard) δ 3.83 (3 H, s), 6.87 (1 H, d, J = 1.8 Hz), 7.12 (1 H, d, J = 1.8 Hz). The extremely hydroscopic nature of this compound prevented a satisfactory elemental analysis.

Anal. Calcd for $C_7H_{11}N_4O_2Cl$: C, 38.45; H, 5.07; N, 25.63. Found: C, 37.93; H, 5.38; N, 24.89.

Registry No.—**4**, 24250-74-6; **4** 2HCl, 27557-44-4; **4** Me ester 2HCl, 27557-45-5; **6** acetate, 27557-46-6; **7** HCl, 27557-47-7; **9** sulfate, 27557-48-8; **9** HCl, 27617-87-4; **10** HCl, 27557-49-9.

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The Stereoselective Total Synthesis of Racemic Fukinone

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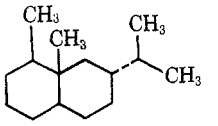
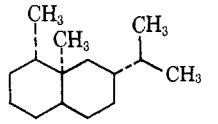
Two synthetic approaches to racemic fukinone, a sesquiterpene ketone of the eremophilane–valencane type, are described. Both utilize a decalone intermediate **12** synthesized from the known unsaturated alcohol **7** *via* acetylation, allylic oxidation, conjugate methylation, Wolff–Kishner reduction, and oxidation. The stereochemically crucial step of this sequence, conjugate methylation of enone **9**, was effected cleanly with lithium dimethylcopper(I). A reaction sequence involving a novel reduction–fragmentation of a β,γ-epoxynitrile (**15** → **16**) failed for lack of a suitable method for oxidizing the resulting allylic alcohol **16**. An alternative route involving addition of isopropenyllithium to the acetoxy ketone **20** and hydrogenolysis of the derived α-acetoxy ketone **23** was accordingly examined. This route led to a mixture of unsaturated ketones which isomerized to racemic fukinone (**17**) upon chromatography.

Considerable effort has been invested over the past several years in the development of rational schemes for the synthesis of sesquiterpenes related to the valencane–eremophilane family.[1] One of the difficulties in designing a synthetic approach to such compounds stems from the need for stereochemically selective methods for introducing the distinctive cis-related vicinal methyl substituents. In the case of fukinone (**17**), a sesquiterpene ketone isolated from the flower stalks of a cultivated variety of *Petasites japonicus* Maxim,[2] the presence of a cis-fused decalin system led us to consider the application of lithium dimethylcopper 1,4 addition[3] to an angularly methylated 1-octal-3-one (*e.g.*, **9**) as a means for achieving this task.[4] This report details the successful execution of that plan and the subsequent chemical transformations leading to totally synthetic fukinone (**17**).[5]



valencane



eremophilane

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