and chloroform for IR spectra were filtered through neutral alumina before use.

Vapor phase chromatographic (VPC) analyses were determined on either a Hewlett-Packard 5750 equipped with a flame ionization detector or a Varian 920 equipped with a thermal conductivity detector using helium as the carrier gas under the indicated conditions. The indicated liquid phase was absorbed on 60–80 mesh Chromosorb W AW DMCS.

Silica gel columns used the 0.05–0.2 mm silica gel manufactured by E. Merck and Co., Darmstadt, Germany. Acidic silica gel refers to Silicar CC-4 special "for column chromatography", sold by Mallinckrodt Chemical Works, St. Louis, Mo. Preparative medium-pressure chromatography was performed using glass columns of the indicated length and diameter with fittings supplied by Laboratory Data Control, Riviera Beach, Fla., and an instrument minipump supplied by Milton Roy Co., St. Petersburg, Fla. (instrumentation designed by R. H. Mueller, those laboratories, and copies are available on request). The columns were packed with silica gel H "for TLC acc. to Stahl" (10–40 μ) manufactured by E. Merck and Co., Darmstadt, Germany. Solvents were degassed under water aspirator vacuum prior to use.

Analytical thin layer chromatography was conducted on 2.5 × 10 cm precoated TLC plates, silica gel 60 F-254, layer thickness 0.25 mm, manufactured by E. Merck and Co., Darmstadt, Germany.

"Dry" solvents were dried immediately prior to use. Ether and tetrahydrofuran (THF) were distilled from lithium aluminum hydride; pyridine, triethylamine, diisopropylamine, *N*-isopropylcyclohexylamine, trimethylchlorosilane (Me_3SiCl), hexamethylphosphoramide (HMPA), and benzene were distilled from calcium hydride; dichloromethane, methyl iodide, and hexane were distilled from phosphorus pentoxide. "Petroleum ether" refers to the "analyzed reagent" grade hydrocarbon fraction, bp 30–60 °C, which is supplied by J. T. Baker Co., Phillipsburg, N.J., and was not further purified.

Standard solutions of *tert*-butyldimethylchlorosilane (*t*-$BuMe_2SiCl$) in hexane (ca. 3.3 M) or HMPA (ca. 1.5 M) were employed.

Reactions were run under an argon atmosphere arranged with a mercury bubbler so that the system could be alternately evacuated and filled with argon and left under a positive pressure.

Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(9) Generously supplied by Dr. G. O. Godtfredsen (Leo Pharmaceutical Co.) and A. Brossi (Hoffmann-La Roche and Co.).
(10) H. Immer and K. Huber, *Helv. Chim. Acta*, **54**, 1347 (1971).
(11) In cases where the products were isolated "by solvent extraction", the procedure generally followed was to dilute the reaction mixture with the indicated solvent or to extract the aqueous solution with several portions of the indicated solvent; then the combined organic layers were washed with several portions of water followed by saturated brine. The organic layer was dried over anhydrous sodium or magnesium sulfate, then filtered, and the solvent was evaporated from the filtrate under reduced pressure (water aspirator) using a rotary evaporator. The use of the terms "base wash" or "acid wash" indicate washing the organic solution with saturated aqueous sodium bicarbonate solution or with dilute aqueous hydrochloric acid, respectively, prior to the aforementioned wash with water.

Carbon-13 Nuclear Magnetic Resonance Studies on a New Antitubercular Peptide Antibiotic LL-BM547β

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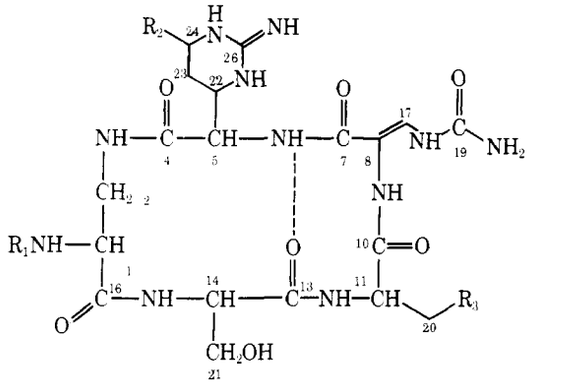
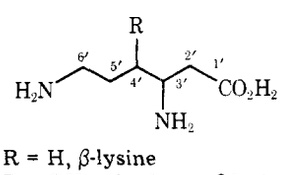
LL-BM547β, a new member of the viomycin family of antibiotics, is described. ^{13}C NMR studies on the antibiotic itself and on a hydrolytic product show that this antibiotic contains the new amino acid N^{β}-methyl-β-arginine as the appendaged amino acid.

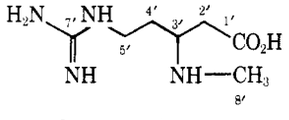
The antitubercular agent viomycin was first described in 1951.[1] Since that time other members of the same family have been isolated, namely the capreomycins[2] and the tuberactinomycins.[3] The structures of tuberactinomycin N[4] and viomycin[5] have been determined by x-ray crystallography.

A *Nocardia* species, Lederle culture BM547, produces two antibiotic components that belong to this same chemical class. By ^{13}C NMR spectroscopy, in conjunction with hydrolytic studies, LL-BM547β was shown to be I. The novelty of I stems from the fact that the appendaged amino acid is *N*-methyl-β-arginine as opposed to β-lysine or γ-hydroxy-β-lysine in the known members (Table I) of this family. The minor component, LL-BM547α or II, was identified as the known de-β-lysylviomycin.[6]





R = H, β-lysine
R = OH, γ-hydroxy-β-lysine



N^{β}-methyl-β-arginine

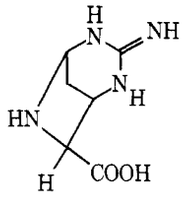
Isolation of I and II. The antibiotic components of culture BM547 in common with most water-soluble basic substances can be removed from the broth filtrate by passage over a weak cation exchange resin in the sodium cycle. The neutralized acid eluate from this resin may be applied directly to a dextran exchanger in the ammonium cycle. I and II may then be selectively eluted from this resin by using an ammonium chloride salt gradient as eluent. The eluate may be desalted over a granular carbon column. The pure antibiotic materials are recovered in about 50–60% yield from carbon columns by elution with 50% aqueous acetone solution. The solid antibiotic is obtained from this eluate by either lyophilization or precipitation with acetone.

Characterization of I and II. These two antibiotics, in common with all the other members of this peptide antitubercular group, have a vinyl urea chromophore which absorbs

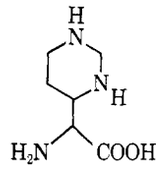
Table I. Summary of the Known Antitubercular Peptide Antibiotics

Antibiotic	R_1	R_2	R_3
LL-BM547β (I)	N^{β}-CH_3-β-arginyl	OH	OH
LL-BM547α (II) delysylviomycin	H	OH	OH
Viomycin	β-Lysyl	OH	OH
Capreomycin	β-Lysyl	H	H
Tuberactinomycin A	γ-OH-β-lysyl	OH	OH
Tuberactinomycin N	γ-OH-β-lysyl	H	OH
Tuberactinomycin O	β-Lysyl	H	OH

Table II. Amino Acid Profile of Antitubercular Peptide Antibiotics[a]



viomycidine



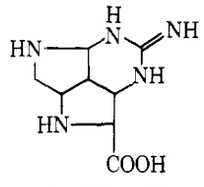
capreomycidine

Antibiotic	Ala	Ser	DAPA	β-Lys	γ-OH-β-Lys	N^β-CH_3-β-Arg	Vio	Cap
LL-BM547β (I)		2	1			1	1	
LL-BM547α (II)		2	1				1	
Viomycin		2	1	1			1	
Capreomycin	1	1	1	1				1
Tuberactinomycin A		2	1		1		1	
Tuberactinomycin N		2	1		1			1
Tuberactinomycin O		2	1	1				1

[a] Ala, alanine; Ser, serine; DAPA, diaminopropionic acid; β-Lys, β-lysine; γ-OH-β-Lys, γ-hydroxy-β-lysine, N^β-CH_3-β-arginine; Vio, viomycidine; Cap, capreomycidine.

strongly at 268 nm. Upon addition of base there is a characteristic shift to 281 nm. This chromophore is of great practical use in the recovery and purification of these compounds. The IR curves of members of this family are frequently superimposable and consequently of no use in identifying individual members. TLC on silica gel using such developing systems as 10% ammonium acetate–acetone–ammonia (90:100:0.67) or 7% ammonium bicarbonate–acetone–ammonia (90:100:1) can be used to distinguish among several family members. The spots on these layers are readily detected by UV absorption or by heating following application of ninhydrin spray reagent. The ^{1}H NMR curves of these materials clearly illustrate some characteristic markers but they are not generally useful in structural elucidation. The vinyl proton of the α,β-unsaturated urea chromophore is observed in the spectra of all members as a singlet at about δ 8.0. The spectra of I, II, and viomycin all exhibit sharp, distinct signals at δ 5.22 and 5.04 which may be attributed to protons at positions C_{24} and C_5 of the arbitrarily numbered diagram shown above. In the spectrum of I a sharp singlet at δ 2.70 for an *N*-methyl group is diagnostic.

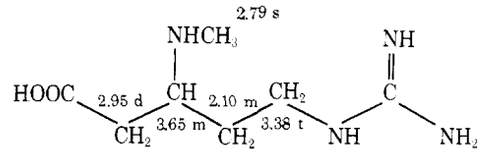
Acidic Hydrolysis. Table II shows the amino acid profile of members of this family of antibiotics. In addition to the indicated amino acids each member also yields 1 mol of ammonia, carbon dioxide, and urea during hydrolysis. Examination of the acid hydrolysate of I by TLC on silica gel revealed the presence of serine and diaminopropionic acid and a material which gave a yellow reaction with ninhydrin. This latter material has been shown to be viocidic acid[7] and the



viocidic acid

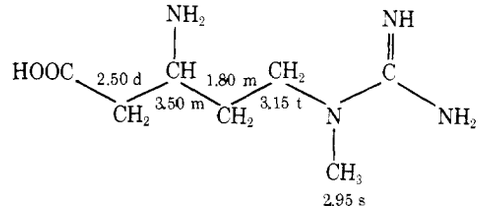
structure shows its relationship with proline, thus accounting for the yellow reaction with ninhydrin. It is an artifact of secondary hydrolysis due to the reactivity of the guanidinocarbinol group.

On silica gel thin layers the Sakaguchi reagent did not react with any product of the hydrolysis. However, TLC of the hydrolysate on cellulose followed by treatment with Sakaguchi reagent showed the presence of two strongly positive materials. One of these is presumably viomycidine. Separation of



N^β-methyl-β-arginine hydrochloride

these compounds in preparative quantities proved to be tedious. The more polar of the two compounds was recovered and shown by ^{1}H NMR and ^{13}C NMR to be N^β-methyl-β-arginine hydrochloride with a specific rotation in water of +20°. The ^{1}H NMR data of this compound are detailed below as are those of the model compound, blastidic acid, obtained by hydrolysis of the antibiotic blasticidin.[8]



blastidic acid

Blasticidic acid is known to be Sakaguchi negative[9] and since the new amino acid is strongly positive, the *N*-methyl substituent must be on the β-amino group.

^{13}C NMR Studies. ^{13}C NMR spectroscopy is by far the most effective diagnostic technique for the characterization of individual members of this antibiotic family. We obtained 25.2-MHz pulsed FT ^{13}C NMR spectra on five members, namely I, II, viomycin, capreomycin, and tuberactinomycin A.[10] The unequivocal structure models provided by the x-ray work already mentioned[4,5] are crucial to the effective use of data from these spectra. Multiplicities of the various carbon atoms in I and viomycin were determined by off-resonance spin decoupling. All the assignments are given in Tables III and IV and in the following discussion the rationale for these assignments is outlined.

Assignment of Carbons in the Macroring. The arbitrary numbering of the various positions in the macroring and in the side chains is shown in the diagram above. Carbons 1 and 2 with doublet and triplet multiplicities, respectively, present in the spectra of all five compounds are best assigned by a process of elimination. In the spectra of four of the antibiotics, that of capreomycin being the exception, four of the carbonyl carbons are bunched between 170.85 and 173.38 ppm. The

Table III. ^{13}C NMR Data on C Atoms of Macroring in I, II, and Related Compounds

Carbon no.	I[a]	II[b]	Viomycin[c]	Capreomycin[d]	Tuberactinomycin A[e]
1	52.83 (d)	51.85	52.78 (d)	51.92	52.83
2	41.13 (t)	40.44	41.23 (t)	40.28	41.27
4	172.6 (s)	172.57	172.57 (s)	172.76	172.74
10	173.40 (s)	173.19	173.38 (s)	176.29	173.47
	55.06 (d)	55.23	55.06 (d)	55.66	55.01
5, 11, 14	55.32 (d)	55.61	55.32 (d)	56.23	55.25
	52.02 (d)	57.06	57.07 (d)	11 at 54.15	57.02
7	167.90 (s)	167.86	167.90 (s)	168.0	168.0
8	105.74 (s)	105.76	105.78 (s)	105.90	105.60
13	171.30 (s)	171.35	171.26 (s)	172.00	170.85
16	171.60 (s)	168.01	172.06 (s)	176.6	171.45

[a] Registry no., 61394-76-1. [b] Registry no., 51628-30-9. [c] Registry no., 32988-50-4. [d] Registry no., 61394-77-2. [e] Registry no., 33103-21-8.

Table IV. ^{13}C NMR Data on Side Chain Carbons of I, II, and Related Antibiotics

Carbon no.	I	II	Viomycin	Capreomycin	Tuberactinomycin A
17	135.58 (d)	135.62	135.53 (d)	135.79	135.70
19	154.26 (s)	156.32	154.26 (s)	155.32	154.35
20	59.86 (t)	59.88	59.88 (t)	18.86	59.88
21	63.31 (t)	63.35	63.35 (t)	68.33	63.23
22	46.83 (d)	46.90	46.88 (d)	49.20	46.83
23	29.93 (t)	30.09	30.09 (t)	23.53	30.14
24	70.83 (d)	70.84	70.87 (d)	49.83	70.87
26	157.20 (s)	156.96	157.00 (s)	157.0 (b)	157.26
1′	171.6 (s)		171.65 (s)	172.0	171.79
2′	34.87 (t)		37.03 (t)	36.93	31.02
3′	54.69 (d)		49.18 (d)	49.26	53.52
4′	29.93 (t)		23.69 (t)	23.59	68.96
5′	38.32 (t)		29.78 (t)	29.77	37.64
6′			39.82 (t)	39.77	34.82
7′	157.5 (s)				
8′	31.28 (q)				

macroring H bonding between the C_{13} carbonyl and the amide proton of position 6 as detected by x-ray work is certain to be present in all five compounds; hence the lowest values around 171 ppm are assigned to C_{13}. The high value near 173 ppm in I, II, viomycin, and tuberactinomycin A is replaced by a still higher value of 176.26 ppm in capreomycin. These values are assigned to C_{10} since in the first four compounds there is the high probability of H bonding between the hydroxymethyl group of C_{11} and the C_{10} carbonyl to form a six-membered ring. No other amide carbonyl is suitably situated for this kind of bonding. The high value in capreomycin is then explained since alanine replaces serine in this portion of the macroring. The value 172.6 is common to all five antibiotics; consequently this is assigned to C_4, which is the position least likely to be affected by the variations between the compounds. Compound I, viomycin, capreomycin, and tuberactinomycin A all show a signal close to 172 ppm which is lacking in the spectrum of II. These values are assigned to C_{16} since II has a free amino group attached to the adjacent C_1 and hence in this compound this signal is shifted to 168 ppm (β shift). The singlet near 168 ppm common in the spectra of all five compounds belongs to the conjugated carbonyl at C_7.[11] The signal at 105.8 ppm with similar characteristics is clearly the conjugated olefinic C_8. Carbons C_5, C_{11}, and C_{14} all with doublet multiplicity are assigned the values clustered between 55 and 57 ppm without further individual characterization except for C_{11} in capreomycin at the low value of 54.15 ppm due to the lack of the β oxygen function.

Assignment of Carbon Atoms of Side Chains. The vinyl C_{17} and the urea carbonyl C_{19} are readily assigned to the signals at 135.6 and 154.3 ppm, respectively. A triplet carbon peak at 59.8 ppm in each spectrum except that of capreomycin must be assigned to C_{20}. While capreomycin lacks this signal, it has a peak at 18.86 ppm unmatched in the others which belongs to methyl carbon C_{20} in this compound. A peak with triplet multiplicity at 63.3 ppm common to all five spectra is assigned to C_{21}. Carbons C_{22}, C_{23}, and C_{24} are assigned as shown in Table IV since in capreomycin the hydroxy group of C_{24} is missing and the α and β effects of the oxygen atom are observed by comparison of C_{22} and C_{23} in the spectrum of this compound with the corresponding signals in the spectra of the others. The guanidino C_{26} is located at about 157 ppm and is clearly visible in all spectra except that of capreomycin, where it is so broad as to be barely detectable. All of the carbons of the amino acid side chains may be picked out readily since they are all missing in the spectrum of II. With the exceptions of the carbonyl $C_{1'}$, which is around 172 ppm, and the guanidine $C_{7'}$ in I, which is found near 157 ppm, all the others appear as intense peaks between 69 and 22 ppm. Their intensities alone set them apart from other carbons in the spectra in the same range. The assignments of $C_{2'}$ through $C_{5'}$ in I, viomycin, capreomycin, and tuberactinomycin A and $C_{6'}$ in the last three of these compounds are readily made by spectral comparisons taking into account perturbation shifts due to N-methylation in I and α and β effects due to the presence of the γ-hydroxy group in tuberactinomycin A.[12] The one remaining signal at 31.28 ppm with quartet multiplicity in the spectrum of I obviously belongs to the *N*-methyl carbon.

We obtained ^{13}C NMR spectra on the hydrochloride salts of *N*-methyl-β-arginine and β-lysine and the data are shown in Table V. The agreement between the chemical shift values for the free acid salts and the corresponding values in the antibiotics I, viomycin, and capreomycin is quite good as seen by comparison of Tables IV and V.

Studies against *Mycobacterium tuberculosis* in mice showed I to be as effective as either viomycin or streptomycin.

Table V. ^{13}C NMR Data on the Hydrochloride Salts of N^β-Methyl-β-arginine and β-Lysine

Carbon	N^β-Methyl-β-arginine[a]	β-Lysine[b]
1′	174.55 (s)	174.56 (s)
2′	34.60 (t)	36.54 (t)
3′	54.32 (d)	48.63 (d)
4′	29.78 (t)	23.63 (t)
5′	38.28 (t)	29.73 (t)
6′		39.82 (t)
7′	157.64 (s)	
8′	31.22 (q)	

[a] Registry no. (HCl), 61394-78-3. [b] Registry no. (HCl), 37894-33-0.

However, the tolerated dose of LL-BM547β was 32 times less than that of streptomycin or 16 times less than that of viomycin. Other relevant biological data are given below.

Experimental Section

IR spectra were taken on in Infracord spectrophotometer, UV spectra were made using a DU Beckman spectrophotometer with recorder attachment, ^{1}H NMR spectra were made on a Varian A100 instrument, and ^{13}C NMR work was carried out on a Varian XL-100 spectrometer. HPLC was carried out on a Chromatronix Model 3520 equipped with a Schoeffel 770 spectrophotometric detector.

Isolation of I and II. Lederle culture BM547 was grown in a 300-L fermentor in a medium consisting of 2% molasses, 1% glucose, 0.5% Bactopeptone, and 0.1% $CaCO_3$ at pH 7.2. Inoculum was prepared in two steps from an agar slant using a medium of 0.5% tryptone, 0.5% yeast extract, 0.3% beef extract, and 1% dextrose. The tank was agitated by impeller at 190 rpm, aerated at 0.5 v v^{-1} min^{-1}, and allowed to incubate for about 140 h. The broth was filtered using diatomaceous earth and 100 L of filtrate was passed over 1 L of weak cation exchanger[14a] in the Na^+ cycle. The resin was washed with water and with acetate buffer at pH 4.8 until the pH of the effluent was unchanged. The resin was then eluted with 0.36 N H_2SO_4 and the eluate adjusted to pH 6.0 using warm $Ba(OH)_2$ solution. After filtration the solution was concentrated to 1 L and 2 L of acetone was added. The resultant precipitate was recovered and dried to 5.5 g of off-white solid. This solid was taken up in 100 mL of H_2O and charged to 500 mL of dextran resin[14b] in the Na^+ cycle. This column was eluted with a gradient of 0.6–6.0% NH_4Cl in H_2O. Elution was monitored by UV absorption at 268 nm and fractions of 60–65 mL volume were collected. Fractions 45–55 were combined and shown to be very rich in II by TLC on silica gel using the system 7% NH_4HCO_3 (90:100:1). Fractions 56–60 were combined and passed over 120 mL of granular carbon.[14] The carbon was then washed with 1200 mL of H_2O and eluted with 360 mL of 50:50 acetone–H_2O. UV measurement indicated that recovery off the carbon column was 62% of charge. The acetone–water eluate was concentrated to 100 mL of aqueous phase and freeze dried to yield 1.2 g of white solid. The OD of this preparation at a concentration of 25 μg/mL in H_2O at 268 nm was 0.73. For HPLC 20 μg was charged to a silica gel column[14c] and developed using a gradient of 20–50% B in A where A was absolute alcohol and B was 10% NH_4OAc solution with pH adjusted to 8.5 using concentrated NH_4OH. This test indicated that the solid was 97% of a major component with traces of two minor components. The major component was I, $[\alpha]^{25}D$ −33 ± 2° (*c* 0.69, H_2O). Microanalytical elemental analyses values for I, while quite reproducible, were consistently low in the carbon and nitrogen values. A typical analysis gave values such as the following.

Anal. Calcd for $C_{26}H_{45}N_{15}O_{10}\cdot 3HCl$: C, 37.29; H, 5.85; N, 25.10; Cl, 12.61. Found: C, 34.78; H, 5.88; N, 22.34; Cl, 12.58.

Fractions 45–55 above, which were shown to be rich in II, were processed by granular carbon to yield about 300 mg of solid. Cuts such as these from several columns were combined and rechromatographed over dextran resin[14b] in the Na^+ cycle and eluted with a gradient of 0.4–4% NH_4Cl. This column was again monitored by UV. Fraction volumes were 80–85 mL and fractions 34–43 were passed over 80 mL of granular carbon and worked as before to get 300 mg of white solid which by TLC was about 95% II with less than 5% I present. The OD of a 25 μg/mL solution in H_2O was 0.69, $[\alpha]^{25}D$ −28° (*c* 1.07, H_2O).

Anal. Calcd for $C_{19}H_{31}N_{11}O_9\cdot 2HCl$: C, 36.36; H, 4.94; N, 24.56. Found: C, 35.32; H, 5.76; N, 22.34.

As in the case of I, the carbon and nitrogen values are low. The biological activity of II is less than that of I or viomycin.[13]

Hydrolysis of I. Approximately 5.0 g of I was refluxed for 16 h in 200 mL of 6 N HCl. The solvent was evaporated off under reduced pressure and the residue treated with H_2O and evaporated to dryness several times until the sharp odor of HCl was no longer detected. The residual 6 g of oil was taken up in 75 mL of H_2O and passed over 30 g of cation-exchanger of 200–400 mesh in the H^+ form.[14d]

The column was eluted with a gradient of 0.3–4 N HCl and fraction volumes were in the range 40–50 mL. Elution progress was followed by paper chromatography using the system *t*-BuOH–H_2O–HOAc (2:1:1), by TLC on silica gel using 10% NH_4OAc–acetone–concentrated NH_4OH (90:100:0.67), and by cellulose TLC using $CHCl_3$–MeOH–NH_4OH–H_2O (1:4:2:1). Fractions 9–12 contained serine and 15–23 contained diaminopropionic acid (DAPA). Fractions 24–35 contained two Sakaguchi-positive materials as detected by cellulose TLC and one ninhydrin-yellow material as detected by paper chromatography. These fractions were combined and charged to 23 g of cation exchange resin[14a] (16–50 mesh) in the H^+ cycle. The column was eluted with H_2O and the eluate charged to 75 mL of dextran resin[14b] in the NH_4^+ form. Elution was carried out using a gradient of 0.5–3% ammonium formate. Fraction volumes were in the range 35–40 mL. Fraction 44–51 contained the Sakaguchi-positive material and these were freeze dried to about 500 mg of residue which was charged to 20 g of cation exchanger in the H^+ form (100–200 mesh).[14e] Elution was carried out using a gradient of 0.1–1.0 N HCl. Fraction volumes were 15–18 mL and the Sakaguchi-positive material appeared in fractions 20–23. Fraction 23 was evaporated to dryness several times, then reconstituted in H_2O and freeze dried to yield 20 mg of N^β-methyl-β-arginine hydrochloride salt which by TLC was essentially pure, $[\alpha]^{25}D$ +20 ± 2° (*c* 0.75, H_2O).

The material gave a clean ^{1}H NMR spectrum (D_2O): δ 2.10 m (2 H, C_3 methylene), 2.79 s (3 H, NCH_3), 2.92 d (2 H, C_2 methylene), 3.38 t (2 H, C_4 methylene), 3.65 m (1 H, C_3 methine).

Fractions 20–22 described above were combined and concentrated and chromatographed over 100 g of cellulose powder[14f] using the upper phase of $CHCl_3$–MeOH–concentrated NH_4OH (2:1:1) as the stationary phase and the lower phase as developing solvent. Fraction volumes of 60–65 mL were collected and fraction 95 contained about 200 mg of essentially pure *N*-methyl-β-arginine. Some of this material was converted to the hydrochloride salt and used for ^{13}C NMR work to get clean spectra. In our hands the material failed to crystallize.

Hydrolysis of II. About 100 mg of II was hydrolyzed in 10 mL of 6 N HCl at 100 °C for 16 h. At the same time a similar hydrolysis was carried out on viomycin. Chromatography of the acid-free hydrolysates showed the presence of serine, DAPA, and β-lysine in viomycin, while the hydrolysate of II showed only serine and DAPA.

Acknowledgments. We wish to thank Mr. A. C. Dornbush and associates for biological assays, Dr. J. Thomas and Mrs. N. Kuck for in vivo testing data, Mr. L. Brancone and associates for microanalytical data, Mr. W. Fulmor and associates for spectral and rotation data, Mr. A. J. Shay and Mr. M. Dann and associates for large-scale fermentations and recoveries, and Dr. H. Tresner for advice on nomenclature of microorganisms. Finally we thank Dr. W. Curran for the ^{13}C NMR data on β-lysine.

Registry No.—I 3HCl, 61473-86-7; II 2HCl, 61473-87-8.

References and Notes

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reactive guanidino carbinol or amino groups.

(10) Viomycin and capreomycin are commercially available as the sulfates. Tuberactinomycin A was isolated from a *Streptoverticillum* species (Lederle culture B0471). It is worth mentioning that hydrochloride or sulfate salts of these compounds give good ^{13}C NMR spectra whereas the carbonate salts give poorer quality spectra.

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(13) For comparison purposes the minimal inhibitory concentrations in μg/mL of I, II, and viomycin are given against two bacterial species in the agar dilution assay. *Mycobacterium smegmatis* ATCC 607: I, 2.5; II, 25; viomycin, 2.5. *Klebsiella pneumoniae:* I, 10; II, 100; viomycin, 25.

(14) (a) IRC-50 resin from Rohm and Haas Co., Philadelphia, Pa. (b) CM-Sephadex C-25 obtainable from Pharmacia Fine Chemicals, Inc., Piscataway, N.J. (c) Vydac column Mo. SS-2-500 A-107 from Spectra Physics, Santa Clara, Calif. (d) AG 50W-X4 from Bio-Rad Laboratories, Richmond, Calif. (e) Amberlite CG-50 is supplied by Mallinckrodt, St. Louis, Mo. (f) Cellulose CF11 from Whatman, Inc., Clifton, N.J.

Facile Synthesis of Amino Acid and Peptide Esters under Mild Conditions via Cesium Salts

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A facile procedure for the preparation of a wide variety of esters derived from protected amino acids and peptides under mild conditions is described. The carboxylic acid to be esterified is first titrated to pH 7 with cesium carbonate or cesium bicarbonate and the neutral salt obtained is then allowed to react with different alkyl halides to form the corresponding esters. The reaction is simple, easily scaled up, and proceeds without observable racemization. Many amino acid and peptide esters that might be difficult to prepare by other methods have been made by this method. The usefulness of this procedure is further demonstrated by the synthesis of methionine-enkephalin.

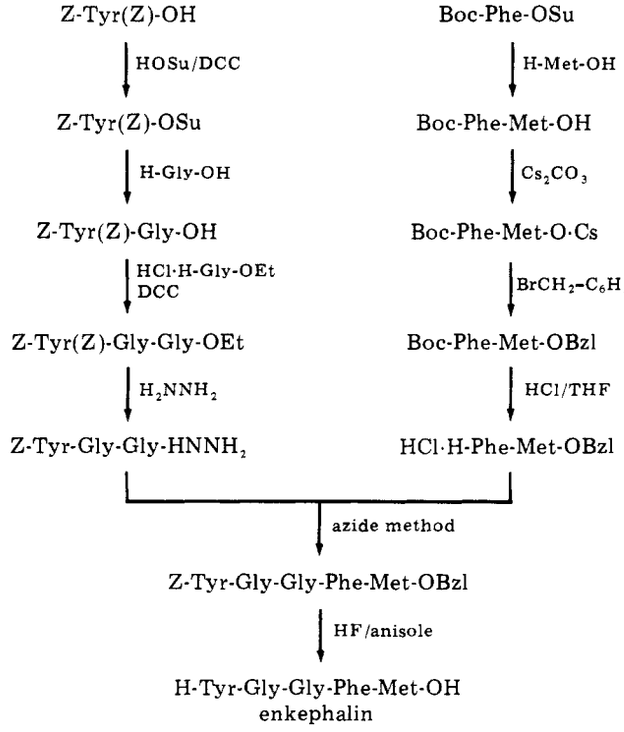
Many useful processes for esterification of carboxylic acids have been reported in the literature.[1-10] However, a great need still exists for a versatile and facile procedure to prepare esters under mild conditions. Such a procedure should be applicable to compounds that are sensitive to acidic, basic, or thermic conditions without depending on exotic expensive reagents. Recently, in an effort to reduce the number of side reactions encountered in solid-phase peptide synthesis,[11] a method for total esterification of Merrifield resins (chloromethylated copolystyrene–1% divinylbenzene) with the cesium salts of Boc-amino acids[12] was investigated.[13] The reaction was found to proceed rapidly and quantitatively under mild conditions. The process has since been satisfactorily utilized to prepare β-phenacyl aspartate[14] and Boc-valyl-4-(oxymethyl)phenylacetic acid.[15] Preparation of other polymer-bound benzyl esters[13,16] and polymer-bound α-methylphenacyl esters has also been described.[17] In the following, we describe the application of this principle to the synthesis of protected amino acid and protected peptide esters in solution.

Reaction of suitably protected amino acid or peptide cesium salts with alkyl halides yielded the desired esters readily under neutral conditions at room temperature. No racemization was observed during this process. The reactions were easily carried out and the yields were generally very high.

In Table I, α-carboxylic esters of amino acids and peptides prepared in this study are listed. The general procedure is as follows. The carboxylic acid to be esterified is first converted into its cesium salt by titration to neutrality with aqueous $CsHCO_3$ or Cs_2CO_3. The latter reagent is more economical. After evaporation to dryness the neutral salt is treated with an alkyl halide in DMF to form the corresponding ester. For example, the benzyl esters I–XII (Table I) were obtained within a short period of time by stirring equivalent amounts of benzyl bromide and the cesium salts of Boc-amino acids or Boc-peptides in DMF at room temperature.

The combination of amine protection by the *tert*-butyloxycarbonyl group and carboxyl protection by the benzyl ester has been the most widely used tactic in peptide synthesis.[18-20] The scope and versatility of this useful approach is now further enhanced by the facile introduction of the benzyl ester group into protected peptides, as shown in Scheme I, with a synthesis of methionine-enkephalin as an example. Thus, Boc-Phe-Met-OH was readily converted in high yield into its benzyl ester Boc-Phe-Met-OBzl (VII) by the cesium salt

Scheme I. Synthesis of H-Tyr-Gly-Gly-Phe-Met-OH (Enkephalin)



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