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# Total Synthesis of *dl*-Anisomycin[1]

Sadao Oida and Eiji Ohki

*Central Research Laboratories, Sankyo Co., Ltd.*[2]

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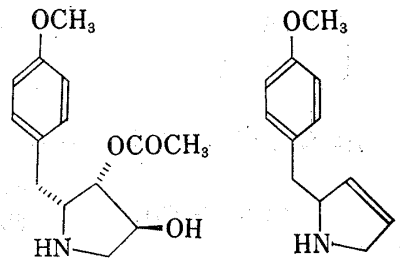
Epoxidation of 2-(*p*-methoxyphenylmethyl)-1-benzyloxycarbonyl-3-pyrroline (**39**) with pertrifluoroacetic acid gave 3α,4α-epoxide (**40**) and 3β,4β-epoxide (**41**). Acetolysis of **41** predominantly yielded 4α-acetoxy-3β-hydroxypyrrolidine (**43**), which was converted into the α-epoxide (**40**) by mesylation and successive treatment with bases. Treatment of the α-epoxide (**40**) with trifluoroacetic acid, followed by acetylation and removal of the protecting groups gave 4α-acetoxy-3β-hydroxypyrrolidine (**47**) and *dl*-anisomycin (**1**). **1** showed superimposed infrared and nuclear magnetic resonance spectra with those of natural anisomycin and exhibited one-half the activity against *Candida albicans*. In addition, the epoxide-cleavage reaction of these 3,4-epoxypyrrolidines was discussed.

The antibiotic anisomycin,[3,4] a fermentation product of various species of *Streptomyces*, has been shown to have widespread activity against pathogenic protozoa and fungi, notably *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Candida albicans*,[4,5] and has already been used for the treatment of amebic dysentery.[6] Chemical studies[7] on anisomycin and successive X-ray investigations[8] have elucidated its structure as 3α-acetoxy-2α-(*p*-methoxyphenylmethyl)-4β-hydroxypyrrolidine (**1**) as shown in Chart 1. Recently, Wong reported that the absolute configuration of anisomycin should be 2R, 3S, 4S from its chemical correlation with a transformation product obtained from natural L-tyrosine.[9] Moreover, Butler demonstrated that tyrosine, glycine, methionine, and acetate are precursors for the biosynthesis of this antibiotic.[10] The present paper describes a total synthesis of *dl*-anisomycin from tyrosine.




1 2

Chart 1

1) Preliminary details of this work have been published: S. Oida and E. Ohki, *Chem. Pharm. Bull.* (Tokyo) **16**, 2088 (1968).
2) Location: *Hiromachi, Shinagawa-ku, Tokyo.*
3) B.A. Sobin and F. Tanner, Jr., *J. Am. Chem. Soc.*, **76**, 4053 (1954).
4) F.W. Tanner, Jr., J.E. Lynch, and J.B. Routin (Chas, Pfeizer & Co., Inc.), U.S. Patent 2691618 (1954).
5) J.E. Lynch, A.R. English, H. Bauck, and H. Deligianis, *Antibiot. Chemotherapy*, **4**, 844 (1954); F.W. Tanner, Jr., B.A. Sobin, and J. Gardocki, *Antibiotics Ann.*, 1954/1955, 809; J.E. Jynch, A.R. English, B.J. Bamforth, and D. Geockeritz, *ibid.*, 1954/1955, 813; W.W. Frye, J.G. Mule, and C. Swartzweder, *ibid.*, 1954/1955, 820; J.E. Lynch, E.C. Holley, and J.E. Margison, *Antibiot. Chemotherapy*, **5**, 508 (1955).
6) A.B. Cue and J.G.H. Diaz, *Rev. Invest. Biol. Univ. Guadalajara*, **1**, 94 (1951); G.G. Miranda and E. Urbina, *ibid.*, **1**, 95 (1951); J.A. Portilla, *ibid.*, **1**, 95 (1951); A.A. Plata, H.B. Zapata, and V.A. Munoz, *ibid.*, **1**, 96 (1951).
7) J.J. Beereboom, K. Butler, F.C. Pennington, and I.A. Solomons, *J. Org. Chem.*, **30**, 2334 (1965).
8) J.P. Schaefer and P.J. Wheatley, *Chem. Commun.* **12**, 578 (1967); *idem*, *J. Org. Chem.*, **33**, 166 (1968).
9) C.M. Wong, *Can. J. Chem.*, **46**, 1101 (1968).
10) K. Butler, *J. Org. Chem.*, **31**, 317 (1966).

In our previous paper,[11] we reported that oxidation of 1-acyl-3-pyrroline with pertrifluoroacetic acid afforded 1-acyl-3,4-epoxypyrrolidine in a good yield and the latter could be converted into various 3,4-*trans*-disubstituted pyrrolidines by successive treatment with nucleophiles. Accordingly, 2-(*p*-methoxyphenylmethyl)-3-pyrroline (**2**), which would be transformable into anisomycin by the analogous series of reactions, was chosen as an intermediate for the synthesis of anisomycin. The commercially available sample of tyrosine which was supplied for the preparation of **2** was furnished as a DL-form or a not-expensive L-form.

Following the procedure of McKinney, *et al.*,[12] cyanoethylation of DL-tyrosine in the presence of bases gave DL-N-(2-cyanoethyl)tyrosine (**3a**), mp>270°, and successive treatment of **3a** with hydrogen chloride in ethanol yielded ethyl ester of DL-N-(2-ethoxycarbonylethyl)-tyrosine (**4a**), mp 71—72.5°, in 85% yield from DL-tyrosine. Similarly, cyanoethylation of L-tyrosine, followed by treatment with acids in ethanol gave ethyl ester of L-N-(2-ethoxycarbonylethyl)tyrosine (**4b**), mp 46—47°. First, we attempted O-methylation of **4b** with diazomethane, but the result was not satisfactory; treatment of **4b** with excess amount of diazomethane in ether afforded asyrup whose thin-layer chromatogram revealed three spots

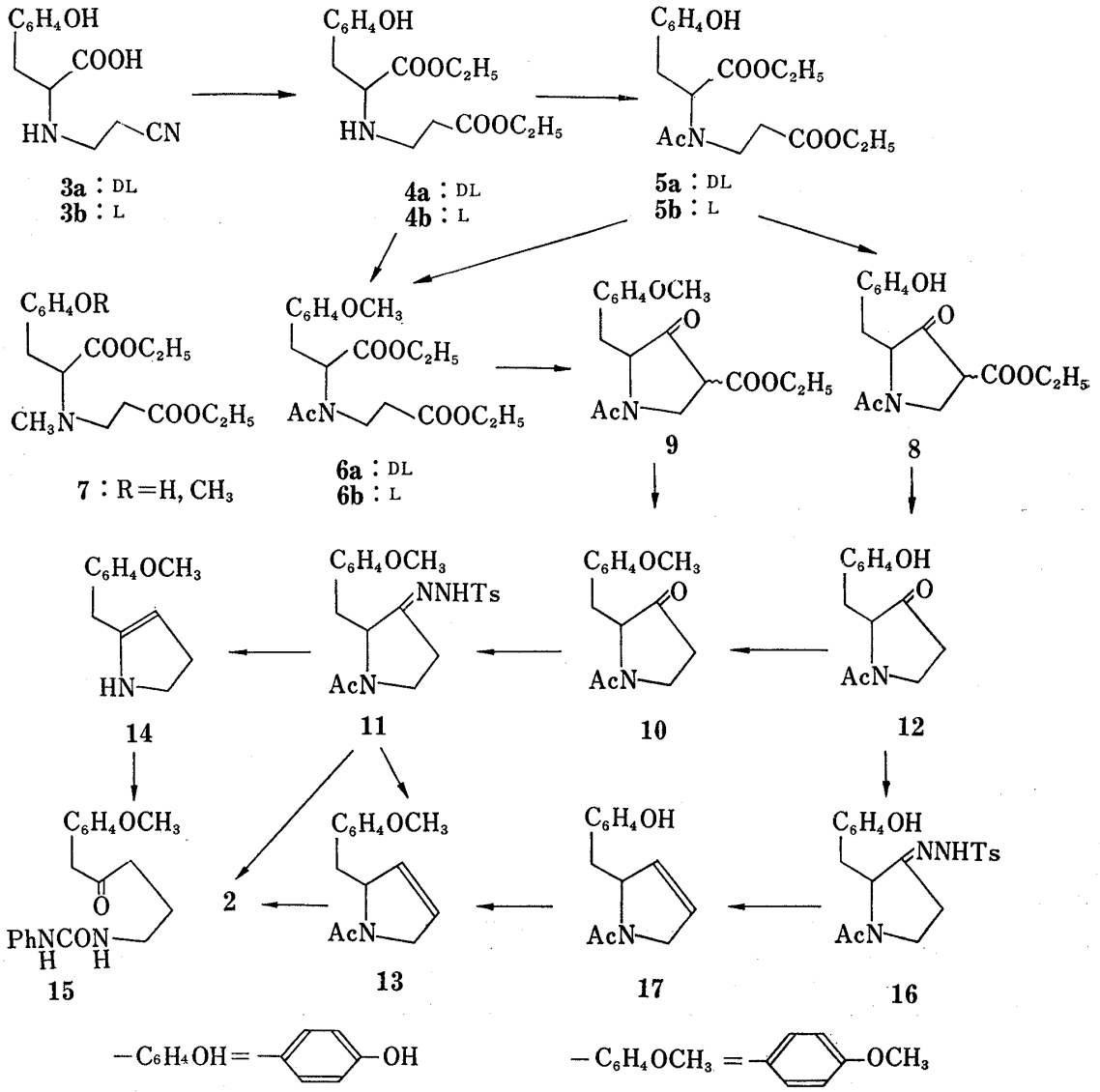



Chart 2

11) S. Oida and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1637 (1968); *idem*, *ibid.*, **17**, 980 (1969).
12) L.L. McKinney, W.H. Uhing, E.A. Setzdorn, and J.C. Cowan, *J. Am. Chem. Soc.*, **72**, 2599 (1950); *idem*, *ibid.*, **74**, 5183 (1952).

along with the unchanged material. As separation of each component from the mixture was found to be difficult, the mixture was acetylated, without purification, with acetic anhydride in acetic acid, yielding an inseparable mixture of ethyl ester of L-N-acetyl-N-(2-ethoxycarbonylethyl)tyrosine (**5b**) and its O-methyl derivative (**6b**).[13] Dieckmann cyclization reaction of the mixture of **5b** and **6b** was carried out with sodium hydride in benzene, yielding a crystalline 1-acetyl-4-ethoxycarbonyl-2-(*p*-hydroxyphenylmethyl)pyrrolidin-3-one (**8**), mp 189—193°, in 21% yield from **4b**, along with its non-crystalline crude O-methyl derivative (**9**). The infrared spectrum of **8** in Nujol mull exhibited a strong hydroxyl absorption at 3150 $cm^{-1}$ without a five-membered carbonyl absorption, indicating that **8** exists in an enolic form in the solid state. The syrupy crude **9** was decarboxylated into the desired 1-acetyl-2-(*p*-methoxyphenylmethyl) pyrrolidin-3-one (**10**), which will be described later in details, but the yield was 28% from **4b**.

As mentioned above, the route starting from the O-methylation of **4b** with diazomethane was found to be unsuitable because of the complexity of the resulting products and the low yield of the desired compound (**10**). Subsequently, we turned to an initial N-acetylation of **4a** or **4b** and subsequent O-methylation of the resulting N-acetate. Treatment of **4a** (DL-form) with acetic anhydride in methanol afforded an N-acetate (**5a**), mp 126—128°, quantitatively, and successive treatment of **5a** with dimethyl sulfate in acetone, in the presence of potassium carbonate gave ethyl ester of DL-N-acetyl-N-(2-ethoxycarbonylethyl)-O-methyltyrosine (**6a**) as a colorless syrup. The yield of **6a** was 77.5% from **4a**. Similarly, treatment of **4b** (L-form) with acetic anhydride in methanol, followed by etherification of the resulting N-acetate (**5b**), mp 94.5—95°, afforded **6b** in a good yield. Dieckmann cyclization reaction of **6a** or **6b** with sodium hydride in benzene gave the corresponding β-ketoester (**9**) as a syrup, which exhibited positive ferric chloride test, in a good yield.[14] **9** was decarboxylated with hydrochloric acid in acetic acid to yield the desired and optically inactive 1-acetyl-2-(*p*-methoxyphenylmethyl)pyrrolidin-3-one (**10**) as a syrup, in a good yield. **10** was purified as its tosylhydrazone (**11**), mp 218° (decomp.). The yield of **11** from **6a** or **6b** was 72%. Moreover, decarboxylation of the afore-mentioned demethyl β-ketoester (**8**) afforded a crystalline pyrrolidin-3-one (**12**), mp 207—208° (decomp.), whose treatment with diazomethane gave **10**.

Bamford-Stephens reaction[15,16] of the tosylhydrazone (**11**) with potassium hydroxide in diethylene glycol for 1.5 hours gave 1-acetyl-2-(*p*-methoxyphenylmethyl)-3-pyrroline (**13**), bp 133—134° (0.1 mmHg), along with 2-(*p*-methoxyphenylmethyl)-2-pyrroline (**14**), bp 125—130° (0.7 mmHg). The structure of the latter (**14**) was verified by the fact that **14** was easily converted into γ-ketophenylurea (**15**), mp 134.5—135°, when treated with phenylisocyanate. Formation of **13** and **14** by the short-time treatment with bases suggested that formation of the double bond occurs at the 2-3 position and at the 3-4 position without selectivity and, further, the N-acetyl group of 2-pyrroline was more easily removed with bases. On the other hand, a long-time treatment of **11** with bases gave the desired 2-(*p*-methoxyphenylmethyl)-3-pyrroline (**2**), bp 113—115° (0.4 mmHg) in 47% yield. The isomeric 2-pyrroline (**14**) could not be detected by vapor-phase chromatography of the reaction product. This

13) As by-products of the etherification, tertiary amine derivatives, which were not acetylated, were obtained in a small amount. The amines could not be charaterized, but would be assignable as N-methyl and N,O-dimethylderivatives (**7**) by their nuclear magnetic resonance spectrometry and in consideration of the analogous examples. *Cf.* E. Abderhalden and E. Schwab, *Z. Physiol. Chem.*, **148**, 20 (1925).

14) The β-ketoester (**9**) obtained from **6a** partly crystallized. Either the crystals, mp 91—94°, or the syrupy product was converted into the same **10** by decarboxylation.

15) V. Carelli and F. Morlacchi, *Ann. Chim.* (Rome), **54** (2), 1291 (1964); T. Mizoguchi, *Chem. Pharm. Bull.* (Tokyo), **9**, 818 (1961).

16) A new olefin formation reaction from tosylhydrazone with methyllithium in ether at room temperature was also attempted on **11**, but without success. *cf.* R.H. Shapiro and M.J. Heath, *J. Am. Chem. Soc.*, **89**, 5734 (1967).

fact suggested that the labile isomer (**14**) may decompose by drastic treatment with bases. Thus, by following the route **3**→**4**→**5**→**6**→**9**→**10**→**11**→**2**, the desired intermediate (**2**) was obtained in 23% yield from tyrosine. Moreover, a tosylhydrazone (**16**), mp 219° (decomp.), which was obtained from the demethylpyrrolidin-3-one (**12**), was treated with bases to give a 3-pyrroline (**17**) as crystals of mp 169—173°, which was also converted into **2** with diazomethane.

The nuclear magnetic resonance (NMR) spectra of these pyrrolidine derivatives synthesized here could not be completely characterized; especially, the methyl absorption of N-acetyl group was usually observed split into two singlets in a relative intensity ratio of 2:1—4:1 as shown in the experimental part. Presumably, this phenomenon is due to two

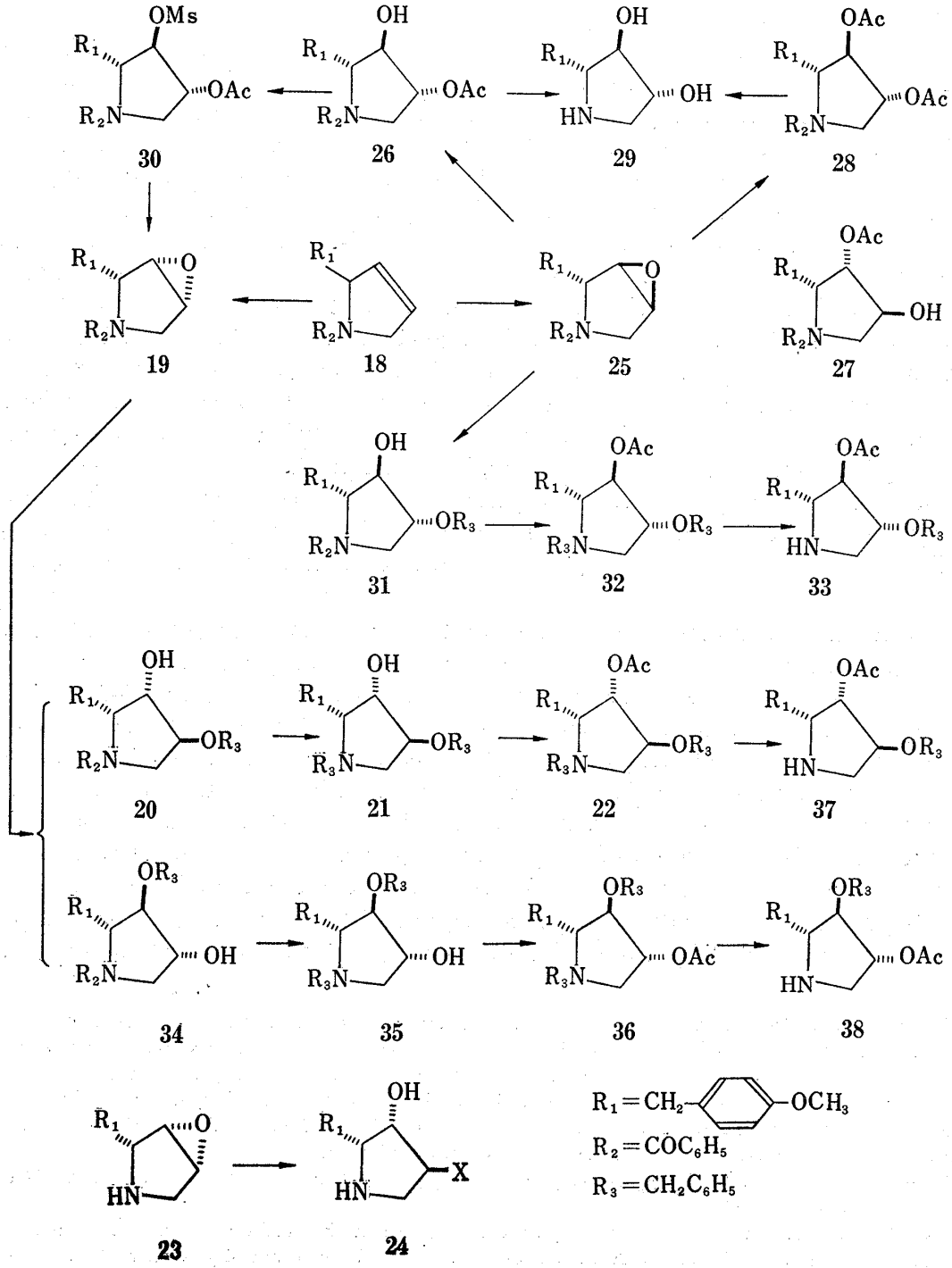



Chart 3

kinds of conformations of the acetyl group, and their environments are shielded differently in view of the magnetic anisotropy of the neighboring 2-*p*-methoxyphenylmethyl group.[17]

Next, it was presumed that the most potential route from **2** to anisomycin (**1**) involves initial protection of the nitrogen in **2** with benzoyl group, epoxidation of the benzoate (**18**), solvolysis of the resulting α-epoxide[18] (**19**) with a benzyloxide ion, reduction of the monobenzyl derivative (**20**) into an N-benzyl derivative (**21**), acetylation of the remaining hydroxyl group, and O,N-debenzylation of the acetate (**22**) into **1**. This route is based on the fact that, as reported previously,[7,8] the ring–opening reaction of the α-epoxide (**23**), which was derived from natural anisomycin, with various nucleophiles proceeded with remarkable stereoselectivity, exclusively giving 3α-hydroxy-4β-substituted derivatives (**24**) and, further, on the consideration that synthesis of anisomycin labile to bases requires mild neutral or acid condition at the final stage which involves removal of the blocking groups.

Epoxidation of the N-benzoate (**18**), which was obtained from **2** with benzoyl chloride, was carried out with pertrifluoroacetic acid in the presence of bases in a following manner. Treatment of **18** with 2.5 equivalents of the reagent at a low temperature afforded the β-epoxide (**25**), mp 109—112°, in 40% yield based on the unrecovered **18**, while epoxidation in boiling dichloromethane gave the β-epoxide (**25**) in 49% yield and its isomeric syrupy α-epoxide (**19**) in 9% yield. These epoxides were separated by repeated chromatography and recrystallization. The NMR spectrum of **19** or **25** did not give any contribution to their chemical structure. These structures were conclusively deduced from the fact that, as will be described later, the α-epoxide (**19**) could be transformed into anisomycin (**1**) whose structure was determined as 2α,3α,4β-substituted pyrrolidine by X–ray analysis.[8] Presumably, the attack of the peracid on the 3–4 double bond of **18** would be effected without selectivity and the lower yield of the α-epoxide (**19**) is ascribed to its unstability to acids. This was supported by an experiment on solvolysis of both epoxides with an acetate ion; the β-epoxide (**25**) was so stable that, after refluxing with acetic acid in the presence of sodium acetate for 8 hours, 20% of the oxide was still recovered. On the other hand, the α-epoxide (**19**) already disappeared from the reaction mixture after only 2 hours. Accordingly, in this epoxidation, the remaining trifluoroacetic acid, which could not be removed with bases, rapidly damages the α-epoxide (**19**), decreasing its yield. Rather at an elevated temperature, which urges the removal of acids more efficiently, the α-epoxide (**19**) still survived during the reaction and was isolated.

The β-epoxide (**25**) was converted into the α-epoxide (**19**) as follows. Treatment of **25** with acetic acid in the presence of sodium acetate gave a syrupy 3β-hydroxy-4α-acetoxypyrrolidine (**26**). In this solvolysis, its possible isomer, 3α-acetoxy-4β-hydroxypyrrolidine (**27**), could not be detected, indicating that this reaction predominantly proceeds under stereospecificity with nucleophilic attack at the 4-position. Long–time treament of **25** with acetic acid gave a 3β,4α-diacetate (**28**) as a by–product. Both **26** and **28** were saponified with bases, giving a crystalline 3β,4α-dihydroxypyrrolidine (**29**), mp 130—131.5°. The monoacetate (**26**) was mesylated in pyridine and successive treatment of the resulting mesylate (**30**) with potassium hydroxide in methanol afforded the α-epoxide (**19**) in a good yield.

Preliminary studies on the ring–opening reaction of these epoxides was carried out as follows. Treatment of the β-epoxide (**25**) with sodium benzyloxide in benzyl alcohol resulted in a predominent formation of one isomer, 3β-hydroxy-4α-benzyloxypyrrolidine (**31**), as a syrup, lithium aluminum hydride reduction of **31**, followed by acetylation afforded 1-benzyl-3β-acetoxy-4α-benzyloxypyrrolidine (**32**) in a good yield. Hydrogenolysis of **32** over palladium charcoal or Adams catalyst was attempted, but the product exclusively obtained was found to be an N-debenzyl-O-benzyl compound (**33**) because its infrared spectrum did not show any

17) F.A.L. Anet, *Can. J. Chem.*, **41**, 883 (1963); H.S. Gutowsky and C.H. Holm, *J. Chem. Phys.*, **25**, 1228 (1956).

18) Following the known formulation of anisomycin (**1**),[9] 2α-*p*-methoxyphenylmethyl group of these trisubstituted pyrrolidines is indicated by dotted line in Chart 3.

hydroxyl absorption, but showed the presence of a benzyloxy group which was also indicated in its NMR spectrum. Hydrogenolysis of **32** ceased at the stage of N-debenzylation and the remaining O-benzyl group resisted further hydrogenolysis. Many attempts for its further reduction were made, but without success. On the other hand, analogous treatment of the α-epoxide (**19**) with sodium benzyloxide gave two benzyloxy compounds, probably the desired 3α-hydroxy-4β-benzyloxypyrrolidine (**20**) and its isomeric 3β-benzyloxy-4α-hydroxypyrrolidine (**34**), which revealed spots separately on thin-layer chromatogram in a relative ratio of *ca.* 1:1 and could be separated by silica gel column chromatography. Either of them was treated with lithium aluminum hydride, giving the corresponding N,O-dibenzyl derivative (**21** or **35**). Hydrogenolysis of its acetate (**22** or **36**) was attempted but, similar with **32**, it was found that both O-benzyl groups resisted hydrogenolysis in spite of facile removal of the N-benzyl group and the corresponding **37** or **38** was obtained in a good yield.

Thus, contrary to our expectation, the first approach to the antibiotic through the ring-opening of the epoxides (**19** and **25**) with a benzyloxide ion was found to be a remote possibility; consequently, a second attempt was made on the introduction of trifluoroacetoxy group, whose protecting group would be easily removed, in place of the benzyloxy group into the 4-position with ring-opening reaction of the α-epoxide. Furthermore, benzyloxy carbonyl group, which is also easily removable by hydrogenation, was chosen to protect the nitrogen function during epoxidation of the double bond in **2**.

**2** formed a syrupy 1-benzyloxycarbonyl-3-pyrroline (**39**) by treatment with benzyloxycarbonyl chloride. By after-treatment as described in the case of **18**, epoxidation of **39** with pertrifluoroacetic acid was carried out and yielded a mixture of the α-epoxide (**40**) and the β-epoxide (**41**) in a relative ratio of 1:5 in 43% yield based on the unrecovered **39**. Each component was separated by repeated chromatography. The major epoxide (**41**) was assigned as the β-epoxide based on the following reactions; removal of the benzyloxycarbonyl group by hydrogenolysis over palladium-charcoal gave a crystalline 3β,4β-epoxypyrrolidine (**42**), mp 83.5—85.5°, which was converted into the afore-mentioned 1-benzoyl-3β,4β-epoxypyrrolidine (**25**) with benzoyl chloride.

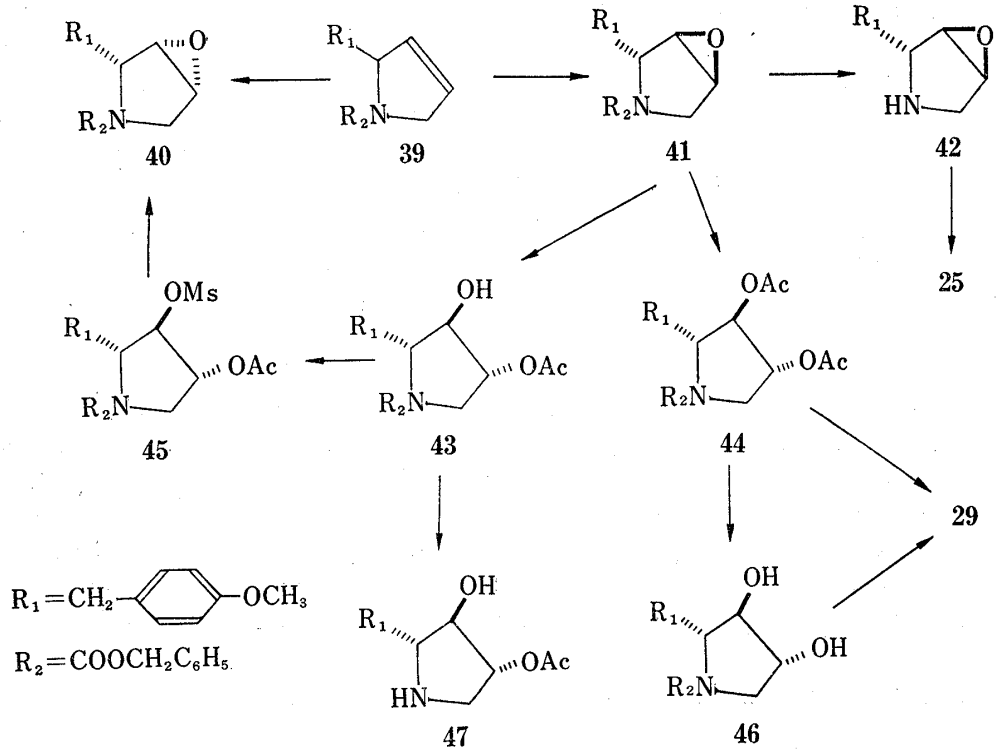



Chart 4

Treatment of **41** with acetic acid in the presence of sodium acetate afforded 3$\beta$-hydroxy-4$\alpha$-acetoxypyrrolidine (**43**), mp 112.5—113°, along with a 3$\beta$,4$\alpha$-diacetoxy derivative (**44**) as a syrup. The former (**43**) was mesylated and the resulting mesylate (**45**) was treated with bases, yielding the minor $\alpha$-epoxide (**40**) in a good yield. Moreover, saponification of the diacetoxy derivative (**44**) with bases gave a 3$\beta$,4$\alpha$-dihydroxypyrrolidine (**46**), mp 101—104°, and its debenzyloxycarbonyl derivative (**29**). The latter was identified with the sample obtained before. Further, hydrogenolysis of **46** gave **29** in a good yield.

Removal of the protecting group from **43** thereby obtained gave 4$\alpha$-acetoxy-2$\alpha$-(*p*-methoxyphenylmethyl)-3$\beta$-hydroxypyrrolidine (**47**), mp 147—148.5°. **47** is isomeric with anisomycin (**1**), but did not show any activity against *Candida albicans*.[19]

Solvolysis of the $\alpha$-epoxide (**40**) with trifluoroacetic acid was carried out in the presence of sodium trifluoroacetate. The resulting product (probably **48** and **49**) could not be characterized because of unstability of its trifluoroacetyl group. Therefore, the product was acetylated with acetic anhydride in pyridine and successively treated with water for removal of the trifluoroacetyl groups. The acetate mixture thereby obtained was chromatographed over silica gel, giving a mixture of monoacetates (probably **43** and **50**) as a syrup in 61% yield. These monoacetates were not successfully separated into each component; therefore, they were hydrogenated over palladium–charcoal for removal of the N-blocking group, affording two kinds of dihydroxypyrrolidine monoacetate which were separated by recrystallization

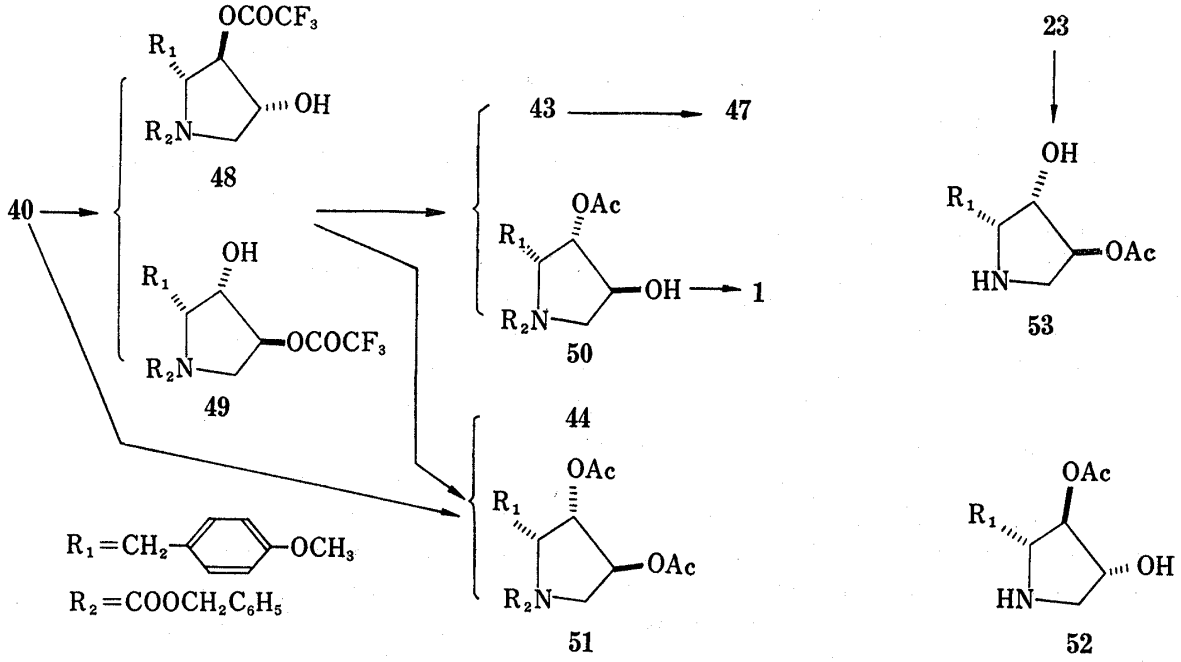



Chart 5

and chromatography as shown in the experimental part. One component (**47**), mp 147—148.5°, was obtained in 17% yield from **40** and was identified with the sample obtained earlier. The other (**1**), mp 118—121°, was obtained in 11% yield from **40** and exhibited superimposable IR and NMR spectra with those of natural anisomycin.[20] The behavior of **1** on thin-layer chromatogram was found to be the same as that of the natural antibiotic.

19) The minimum inhibitory concentration of *dl*-anisomycin (**1**) was assayed against *Candida albicans* as 4 $\mu$g/ml in brain–heart infusion broth and 50 $\mu$g/ml in Sabouraud's agar. The activities of other compounds were compared under the same conditions.

20) The sample of natural anisomycin was supplied by Dr. L. Delcambe, International Center of Information of Antibiotics, Liege, Belgium, to whom we wish to express our appreciation.

Further, the activity[19] of **1** against *Candida albicans* was determined as just one-half of that of natural anisomycin.

In the separation of the monoacetates (**43** and **50**) described above, an inseparable mixture of diacetates was also obtained as a minor product. Its thin-layer chromatogram was agreed with that of 3$\beta$,4$\alpha$-diacetoxypyrrolidine (**44**). The NMR analysis of the mixture indicated the presence of **44** and its possible isomer (**51**) in relative ratio of *ca.* 1:1 as shown in the experimental part. In the case of prolonged acetylation, yield of the diacetate mixture (**44** and **51**) increased, but in the same ratio. This fact would indicate exchange of the trifluoroacetyl group with acetyl group during this acetylation reaction. Further, the formation of **44** and **51** in approximately equivalent amounts suggests that the ring-opening of the $\alpha$-epoxide (**40**) with a trifluoroacetate ion occurs without stereospecificity, yielding **48** and **49** in the same amount. In addition, solvolysis of **40** was carried out in boiling acetic acid and the remaining hydroxyl group was further treated with acetic anhydride in pyridine, affording the same mixture of diacetates (**44** and **51**), whose composition was also determined as 1:1 by NMR analysis. These facts are in parallel with formation of **20** and **34** observed earlier in the ring-opening reaction of the $\alpha$-epoxide (**19**) with a benzyloxide ion. Conclusively, nucleophiles attack the $\beta$-epoxide (**25** or **41**) predominantly at the 4-position, being hindered by the bulky C-2 substituent, while nucleophiles attack the $\alpha$-epoxides (**19** and **40**) without selectivity at the 3- and 4-positions.

As already mentioned, Beereboom, *et al.*[7] observed in the course of the structural study of anisomycin that the 3$\alpha$,4$\alpha$-epoxypyrrolidine (**23**) with unblocked nitrogen, which was derived from natural anisomycin, showed an interesting chemical nature; reaction of **23** with various nucleophiles proceeded smoothly and, in every case, exclusively a single product, 3$\alpha$-hydroxy-4$\beta$-substituted pyrrolidine (**24**) was isolated with predominant introduction of these nucleophiles at the 4-position. Later, Schaefer and Wheatly[8] explained this fact as follows. Conceding that the 2$\alpha$-*p*-methoxyphenylmethyl group prefers to be in an equatorial-like orientation, the lowest-energy transition state for ring opening would be reached by a nucleophilic attack at the 4-position, followed by ideal formation of all coplanarity with 3$\alpha$-axial and 4$\beta$-axial substituents or the likes. This fact is contradictory to the cases of the N-acylated $\alpha$-epoxides (**19** and **40**) whose ring opening reactions occurred without stereospecificity. Presumably, one of the plausible reasons includes that the bulky N-blocking group forces the 2$\alpha$-*p*-methoxyphenylmethyl group to get out of the equatorial-like position and this takes away a limitation on the attack point of nucleophiles.

In the final purification of *dl*-anisomycin (**1**), its new isomer (**52**), mp 100.5—101.5°, was isolated in a low yield. Sufficient characterization of **52** was not made because of scarcity of the sample, but it would be assignable as 3$\beta$-acetoxy-4$\alpha$-hydroxypyrrolidine on the basis that it was also not identical with 3$\alpha$-hydroxy-4$\beta$-acetoxypyrrolidine (*dl*-isoanisomycin) (**53**), mp 144—146°. The latter (**53**) was prepared, following the reported procedure,[7] by hydrogenolysis of **40** and successive treatment of the resulting 3$\alpha$,4$\alpha$-epoxide (**23**) with acetic acid. Presumably, **52** would originate from the $\beta$-epoxide (**41**) persistently contaminated in **40**. These isomers showed no activity against *Candida albicans*.[19]

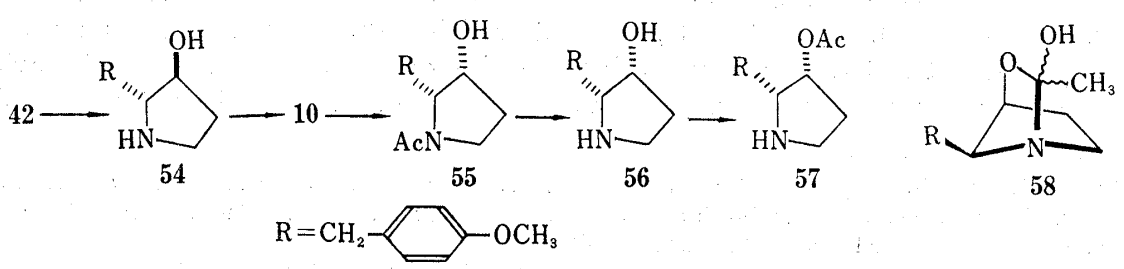



Chart 6

Finally, we would like to mention about deoxyanisomycin. Lithium aluminum hydride reduction of the 3$\beta$,4$\beta$-epoxypyrrolidine (**42**) gave a monohydroxypyrrolidine (**54**), mp 110—111°. N-Acetylation of **54** with acetic anhydride in ethanol followed by oxidation with anhydrous chromic acid gave 1-acetylpyrrolidin-3-one (**10**) which was further characterized as its tosylhydrazone (**11**). This fact indicates that the hydroxyl group in **54** exists at the 3-position and the attack of a hydride ion on **42** was effected at the 4-position. Accordingly, **54** was designated as 3$\beta$-hydroxypyrrolidine. On the other hand, treatment of **10** with sodium borohydride and successive treatment of the resulting 1-acetyl-3-hydroxypyrrolidine[21] (**55**) with bases afforded an isomeric 3$\alpha$-hydroxypyrrolidine (**56**), mp 113—114.5°, in 62% yield. **56** was converted into its N-benzyloxycarbonyl derivative and acetylation of the latter, followed by removal of N-blocking group, afforded a 3$\alpha$-acetoxypyrrolidine (**57**) (deoxyanisomycin) as a colorless syrup.

It was found that these pyrrolidine derivatives synthesized here exhibited no activity[19] against *Candida albicans* except deoxyanisomycin (**57**), which showed 1/10 to 1/20 the activity of *dl*-anisomycin (**1**). This fact is of interest on the basis of Grollman's prediction[23] on the mode of action of anisomycin, suggesting that the stereochemical moiety of the substituents around the 2-position of pyrrolidine may give a substantial contribution to the anisomycin activity.

## Experimental

Melting points are not corrected. Infrared spectra were determined on a Perkin–Elmer Model 221 or a Perkin–Elmer Infracord, and NMR spectra on a Varian A–60 spectrometer. Removal of the solvent *in vacuo* was accomplished by a rotating flash evaporator at 20—30 mmHg, usually at 35—50°. Plates for thin–layer chromatography were prepared with silica gel (E. Merck AG) and visualization of spots was effected by spraying a solution of $NH_4VO_3$ in 50% $H_2SO_4$, followed by heating or by spraying KI–$PtCl_4$ solution.

**Ethyl Esters of DL- (4a) and L-N-(2-Ethoxycarbonylethyl)tyrosine (4b)**——To a stirred solution of 50 g of DL–tyrosine in 300 ml of $H_2O$ was added dropwise a solution of 31 g of KOH in 80 ml of $H_2O$ at below 20° and, to the resulting solution was dropped 15.5g of acrylonitrile at below 10° with stirring. After the addition was completed, the mixture was stirred for 3 hr at room temperature and further for 2 hr at 50—60°. To the cooled mixture was dropped 50 ml of conc. HCl and the crystals formed were collected, yielding 65 g of DL-N-(2-cyanoethyl)tyrosine (**3a**), mp>270°. To the cooled solution of **3a** in 400 ml of EtOH, a stream of HCl gas was passed through for 2 hr and, the mixture was warmed at 60—70° for 2 hr. After the solid ($NH_4Cl$) was filtered off, $Na_2CO_3$ (solid) was added in small portions to the filtrate and the mixture was allowed to stand until the evolution of $CO_2$ ceased. The mixture was basified with dil. $Na_2CO_3$ solution and extracted with benzene. The extract was dried ($Na_2SO_4$) and evaporated to dryness *in vacuo*, leaving a crystalline mass which was recrystallized from benzene–hexane to 72 g (84.5%) of **4a** as needles, mp 71—72 5°. IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3590, 3350, 1732. *Anal.* Cacld. for $C_{16}H_{23}O_5N$: C, 62.12; H, 7.49; N, 4.53. Found: C, 62.08; H, 7.61; N, 4.52.

21) It was found by NMR analysis in $CDCl_3$ that **55** exists as a 1:1 equilibrium mixture of N-acetylpyrrolidine and its cyclic isomer[22] (**58**) as shown in Chart 6. The amide methyl absorption observed as a singlet at 2.04 ppm did not correspond to three protons in comparison with the methoxy methyl absorption at 3.76 ppm and, further, a new absorption corresponding to a methyl group attached with a tertiary carbon was observed at 1.39 ppm as a singlet with an approximate equal intensity. On the other hand, the NMR spectrum of **57** did not show the presence of the corresponding equilibrium like **57**⇄**58**, but reflected the structure of 3-acetoxypyrrolidine.

22) K. Butler, *J. Org. Chem.*, 33, 2136 (1968).

23) Grollman has demonstrated the mode of action of anisomycin in extracts prepared from mammalian cells, yeast, or protozoa, and announced that the antibiotic blocks the transfer of amino acids from the aminoacyl s–RNA to the ribosomes where proteins are assembled, in the way similar to ipecac alkaloids, cycloheximide, and glutarimide antibiotics. Based on configurational and conformational similarities of these compounds, he gave an interesting prediction that the part of the molecule including a secondary amine and a methylene with six–membered ring is essential for inhibition of protein synthesis. *cf.* Grollman, Abstr. 153rd National Meeting of the American Chemical Society, Miami, Fla., April 1967, M2; *J. Biol. Chem.*, 242, 3226 (1967).

In the same manner as above, cyanoethylation of L-tyrosine, followed by esterification, afforded **4b** as needles (from ether–hexane), mp 46—47°. The infrared spectra (IR) of **4a** and **4b** in the solution were identical. *Anal.* Found: C, 61.91; H, 7.54; N, 4.50.

**Ethyl Esters of DL- (5a) and L-N-Acetyl-N-(2-ethoxycarbonylethyl)tyrosine (5b)**——To a solution of 43.4 g of **4a** in 100 ml of MeOH was added 50 ml of $Ac_2O$ with stirring and the mixture was refluxed for 1.5 hr. Concentration of the reaction product *in vacuo* gave crystals which were recrystallized from benzene to 45.5 g of **5a** as prisms, mp 126—128°. IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3590, 3300, 1736, 1641. NMR ($CDCl_3$) $\delta$ ppm: 1.26 and 1.31 (6H, *ca.* 6:1, triplets, $J=7$ cps, $-OCH_2-C\underline{H}_3$), 1.81 and 2.12 (3H, *ca.* 3:1, singlets, $-COC\underline{H}_3$), 2.45 (2H, triplet, $J=7$ cps, $-C\underline{H}_2-COO-$), 4.15 and 4.20 (4H, quartets, $J=7$ cps, $-OC\underline{H}_2-CH_3$), 6.95 (4H, $A_2B_2$-pattern, aromatic), 7.62 and 8.05 (broad singlets, *ca.* 3:1, $-O\underline{H}$). *Anal.* Calcd. for $C_{18}H_{25}O_6N$: C, 61.52; H, 7.17; N, 3.99. Found: C, 61.47; H, 7.13; N, 3.85.

Similarly, **4b** was acetylated to **5b** as prisms (from benzene–hexane), mp 94.5—95°, $[\alpha]_D^{20}$ −124.5° ($c=2$, $CHCl_3$). Its IR and NMR spectra were identical with those of **5a**. *Anal.* Found: C, 61.40; H, 7.16; N, 3.98.

**Ethyl Esters of DL- (6a) and L-N-Acetyl-N-(2-ethoxycarbonylethyl)-O-methyltyrosine (6b)**——A solution of 45.4 g of **5a**, 19.7 g of $Me_2SO_4$, and 20 g of $K_2CO_3$ in 70 ml of acetone was refluxed for 3 hr. When cooled, the reaction mixture was poured into $H_2O$ and extracted with ether. The extract was dried ($Na_2SO_4$) and evaporated *in vacuo*, giving 40.7 g (77.5% from **4a**) of **6a** as a colorless syrup. The product exhibited one spot on a thin-layer chromatogram and was used for the next reaction without further purification. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 1738, 1655. NMR ($CDCl_3$) $\delta$ ppm: 1,25 (6H, triplet, $J=7$ cps, $-OCH_2-C\underline{H}_3$), 1.86 and 2.10 (3H, *ca.* 1:4, singlets, $C\underline{H}_3CO-$) 2.42 (2H, triplet, $J=7$ cps, $-C\underline{H}_2COO-$), 3.81 (3H, singlet, $-OC\underline{H}_3$), 4.14 and 4.21 (4H, quartets, $J=7$ cps, $-OC\underline{H}_2CH_3$), 7.00 (4H, $A_2B_2$-pattern, aromatic).

Analogously, **5b** was treated with $Me_2SO_4$ and gave **6b** as a colorless syrup. The IR and NMR spectra of **6a** and **6b** were identical.

**1-Acetyl-2-(*p*-methoxyphenylmethyl)pyrrolidin-3-one (10) and Its Tosylhydrazone (11)**——A solution of 40.7 g of **6a** in 100 ml of benzene suspended with NaH, which was prepared from 6.4 g of 50% NaH-dispersion by washing with hexane, was gradually warmed to reflux with vigorous stirring. As an exothermic reaction accompanied with gas evolution occurred just before refluxing careful warming and, if circumstances required, powerful cooling were carried out. After refluxing for 2.5 hr, the cooled reaction mixture was carefully acidified with AcOH and shaken. The organic layer was washed with $H_2O$, dried ($Na_2SO_4$) and evaporated to dryness *in vacuo*, leaving 33.5 g of crude **9**. Trituration with ether afforded 18.2 g of crystals, mp 80—91°. Analytical samples were obtained as prisms, mp 91—94°, by recrystallization from benzene. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3500, 3090, 2650, 1711, 1678, 1625. NMR ($CDCl_3$) $\delta$ ppm: 1.26 (3H, triplet, $J=7$ cps, $-OCH_2-C\underline{H}_3$), 1.7—2.2 (3H, multiplet, main peak as singlet at 2.02 ppm, $C\underline{H}_3CO-$), 3.78 (3H, singlet, $-OC\underline{H}_3$), 4.21 (2H, quartet, $J=7$ cps, $-OC\underline{H}_2CH_3$), 2.7—5.2 (multiplet), 6.92 (4H, multiplet, aromatic). *Anal.* Calcd. for $C_{17}H_{21}O_5N\cdot H_2O$: C, 60.52; H, 6.87; N, 4.15. Found: C, 60.57; H, 6.81; N, 4.17.

The crystalline **9** (18.0 g) thus obtained was dissolved in a mixture of 40 ml of AcOH, 40 ml of $H_2O$, and 4 ml of conc. HCl, and the mixture was warmed on a steam bath for 3.5 hr. The reaction mixture was diluted with $H_2O$ and extracted with benzene. The extract was washed with dil. $Na_2CO_3$ solution, dried, and evaporated to dryness *in vacuo*, leaving 13.9 g of crude **10** as a brown syrup. Analytical sample was obtained by distillation to a colorless syrup, bp 180° (0.1 mmHg, bath temp.). IR $\nu_{max}^{liq.}$ $cm^{-1}$: 1758, 1648. NMR ($CDCl_3$) $\delta$ ppm: 1.99 and 2.10 (3H, singlets *ca.* 1:3, $-COC\underline{H}_3$), 3.61 (3H, singlet, $-OC\underline{H}_3$), 6.90 (4H, $A_2B_2$-pattern, aromatic). *Anal.* Calcd. for $C_{14}H_{17}O_3N$: C, 67.99; H, 6.93; N, 5.66. Found: C, 66.65; H, 6.92; N, 5.61.

A solution of crude **10** thus obtained and 10 g of tosyl hydrazide in 80 ml of MeOH was refluxed for 1 hr with stirring. When cooled, 15.9 g of crude **11**, mp 203—207°, was collected. Analytical sample was obtained by recrystallization from EtOH to leaflets, mp 218° (decomp.). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3030, 1624. *Anal.* Calcd. for $C_{21}H_{25}O_4N_3S$: C, 60.71; H, 6.07; N, 10.12. Found: C, 60.39; H, 6.12; N, 9.93.

The syrupy **9** (18.6 g) which remained was treated with acids, as described above, to give 14.3 g of **10** which formed 16.3 g of **11**. Total yield of **11** from **6a** was 72%.

Similarly, Dieckmann cyclization reaction of **6b**, followed by decarboxylation afforded the same optically-inactive **10** which was also characterized as **11**. The yield was almost the same. In this case, the crystalline $\beta$-ketoester (**9**) was not obtained.

**2-(2-*p*-Methoxyphenylmethyl)-3-pyrroline (2)**——The tosylhydrazone (**11**) (35.0 g) was dissolved in 200 ml of diethylene glycol containing 16 g of KOH and the mixture was kept at 140—145° for 24 hr with stirring. The cooled reaction mixture was diluted with 600 ml of $H_2O$ and extracted with two 150 ml portions of benzene. The extract was dried ($Na_2SO_4$) and evaporated to dryness *in vacuo*, and the residue was distilled to yield 7.8 g (49%) of **2** as a colorless syrup, bp 113—115° (0.4 mmHg). IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3350, 1653, 1613, 1512, 1247, 1177, 1032. NMR ($CDCl_3$) $\delta$ ppm: 2.13 (1H, singlet, $-\underset{|}{N}\underline{H}$), 2.72 (2H, doublet, $J=6.5$ cps, $Ar-C\underline{H}_2-$), *ca.* 3.75 (2H, multiplet, $-\underset{|}{N}-C\underline{H}_2-$), 3.79 (3H, singlet, $-OC\underline{H}_3$), 4.20 (1H, multiplet, $-\underset{|}{N}-\underset{|}{C}\underline{H}-$), 5.87 (2H, multiplet, $-C\underline{H}=C\underline{H}-$), 7.01 (4H, $A_2B_2$-pattern, aromatic). *Anal.* Calcd. for $C_{12}H_{15}ON$: C, 76.15; H, 7.99; N, 7.40. Found: C, 78.12; H, 8.28; N, 7.55.

**1-Acetyl-2-(*p*-methoxyphenylmethyl)-3-pyrroline (13) and 2-*p*-Methoxyphenylmethyl-2-pyrroline (14) (Short-time Treatment of 11 with Bases)**——A solution of 36.1 g of **11** and 16 g of KOH in 200 ml of diethylene glycol was kept at 135—140° for 1.5 hr with stirring. The cooled reaction mixture was diluted with 600 ml of $H_2O$ and extracted with two 130 ml portions of benzene. The extract was washed with dil. HCl and $H_2O$, dried ($Na_2SO_4$), and evaporated to dryness *in vacuo*, leaving 11.8 g of a brown syrup which was distilled to yield 11.0 g (55%) of **13** as a colorless syrup, bp 133—134° (0.1 mmHg). IR $\nu_{max}^{liq.}$ 1650 $cm^{-1}$. NMR ($CDCl_3$) $\delta$ ppm: 2.04 and 2.16 (2H, singlets, *ca.* 3.6:1, $C\underline{H}_3CO$–), 2.7—3.25 (2H, multiplet, Ar–$C\underline{H}_2$–), 3.78 (3H, singlet, $C\underline{H}_3O$–), 3.55—4.3 (3H, multiplet, –N–$C\underline{H}_2$–CH=), 4.5—5.15 (1H, multiplet, –N–$C\underline{H}$–CH=), *ca.* 5.70 (2H, multiplet, –$C\underline{H}$=$C\underline{H}$–), 6.95 (4H, $A_2B_2$-pattern, aromatic). *Anal.* Calcd. for $C_{14}H_{17}O_2N$: C, 72.70; H, 7.41; N, 6.06. Found: C, 71.97; H, 7.48; N, 5.94.

The aqueous layer left after extraction of **13** was basified with $Na_2CO_3$ (solid) and extracted with benzene. The extract was washed with $H_2O$, dried ($Na_2SO_4$), and evaporated to dryness *in vacuo*, leaving 6.06 g of a brown syrup which was fractionally distilled to yield 4.9 g (30%) of **14**, bp 125—130° (0.7 mmHg). IR $\nu_{max}^{liq.}$ 1645 $cm^{-1}$. NMR ($CDCl_3$) $\delta$ ppm: 1.5—2.85 (4H, multiplet, –N–$C\underline{H}_2$–$C\underline{H}_2$–), 3.76 (3H, singlet, $C\underline{H}_3O$–), 3.5—4.1 (4H, multiplet, –N$\underline{H}$–, and Ar–$C\underline{H}_2$–C=$C\underline{H}$–), 7.00 (4H, $A_2B_2$-pattern, aromatic). *Anal.* Calcd. for $C_{12}H_{15}ON$: C, 76.15; H, 7.99; N, 7.40. Found: C, 75.47; H, 7.86; N, 7.45.

**14** formed a phenylurea derivative (**15**), mp 134.5—135°, as needles (from 95% EtOH) by treatment of phenylisocyanate in ether. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3370, 3290, 1719, 1649. NMR ($CDCl_3$) $\delta$ ppm: 1.66 (2H, quintet, $J$=7 cps, –CO–$CH_2$–$C\underline{H}_2$–$CH_2$–), 2.44 (2H, triplet, $J$=7 cps, –CO–$C\underline{H}_2$–$CH_2$–), 3.11 (2H, quartet, $J$=6.5 cps, –$CH_2$–$C\underline{H}_2$–N–), 3.57 (2H, singlet, Ar–$C\underline{H}_2$CO–), 3.74 (3H, singlet, $C\underline{H}_3O$–), 5.69 (1H, triplet, $J$=5.5 cps, –$CH_2$–N$\underline{H}$–), 6.96 (4H, $A_2B_2$-pattern, aromatic), 7.25 (5H, multiplet, phenyl), 7.63 (1H, broad singlet, –N$\underline{H}$–). *Anal.* Calcd. for $C_{15}H_{22}O_3N_2$: C, 69.92; H, 6.79; N, 8.58. Found: C, 69.77; H, 6.89; N, 8.91.

**1-Acetyl-4-ethoxycarbonyl-2-(*p*-hydroxyphenylmethyl)pyrrolidin-3-one (8) (Attempted Treatment of 4b with Diazomethane and Successive Acetylation and Dieckmann Cyclization)**——To a solution of 32 g of **4b** in 200 ml of ether was added an excess of $CH_2N_2$-etheral solution and the mixture was allowed to stand for 1.5 hr at room temperature. Evaporation of the solvent gave a syrup, whose thin-layer chromatogram exhibited 3 spots, along with the unchanged **4b**. The syrupy product was dissolved in 12 ml of AcOH containing 40 g of $Ac_2O$ and the mixture was warmed for 30 min on a steam bath. The reaction mixture was evaporated *in vacuo* and the residue (40.3 g) was dissolved in benzene. The benzene solution was washed with 1N HCl and $H_2O$, dried, and evaporated *in vacuo*, yielding 36.8 g of a mixture of **5b** and **6b**. Dieckmann cyclization reaction of this mixture was carried out as described for **6a**, and 6.75 g (21% from **4b**) of a crystalline mass, mp 181—186° (decomp.), was obtained along with 21.5 g of a syrup. The former crystals were insoluble in $H_2O$ or benzene and were recrystallized from a mixture of AcOH and MeOH to **8** as needles, mp 189—193° (decomp.). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3270 (shoulder), 3150, 1697, 1623. NMR ($d_6$-DMSO) $\delta$ ppm: 1.17 (3H, triplet, $J$=7 cps, –$OCH_2$–$C\underline{H}_3$), 1.7—2.1 (3H, multiplet, $C\underline{H}_3CO$–), 2.6—4.8 (8—9H, multiplet), 6.7 (4H, multiplet, aromatic). *Anal.* Calcd. for $C_{16}H_{19}O_5N$: C, 62.94; H, 6.27; N, 4.59. Found: C, 63.15; H, 6.51; N, 4.53.

The remaining syrup was decarboxylated by treatment with hydrochloric acid in AcOH, in the same manner as described before, giving 7.2 g (28% from **4b**) of crude **10** whose purification by fractional distillation was attempted, but the distillate did not show a satisfactory result on elementary analysis. The crude **10** thus obtained (6.2 g) was treated with tosyl hydrazide in MeOH and 5.44 g of **11**, mp 212° (decomp.), was obtained.

**1-Acetyl-2-(*p*-hydroxyphenylmethyl)pyrrolidin-3-one (12) and Its Tosylhydrazone (16)**——A mixture of 5.65 g of the crude **8**, 15 ml of AcOH, 15 ml of $H_2O$, and 1 ml of conc. HCl was warmed on a steam bath for 4.5 hr until the evolution of $CO_2$ gas ceased. The reaction mixture was concentrated *in vacuo* and, after dilution with 5 ml of acetone, the concentrate was stood at room temperature. The resulting crystals were collected, washed with ether, and recrystallized from MeOH to 2.15 g of **12** as small prisms, mp 207—208° (decomp.). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3250, 1762, 1640. NMR ($d_6$-DMSO) $\delta$ ppm: 1.84 and 2.01 (3H, singlets, *ca.* 1:2, $C\underline{H}_3CO$–), 2—4.4 (complex multiplet), 6.76 (4H, multiplet, aromatic), 9.23 (1H, broad singlet, OH). *Anal.* Calcd. for $C_{13}H_{15}O_3N$: C, 66.93; H, 6.48; N, 6.01. Found: C, 66.42; H, 6.58; N, 5.95.

Treatment of **12** with excess of $CH_2N_2$ in ether gave 10 in a good yield.

A mixture of 1.17 g of **12**, 0.93 g of tosyl hydrazide, and 10 ml of MeOH was refluxed for 4 hr and allowed to stand at room temperature overnight, yielding 1.25 g of a tosylhydrazone (**16**), mp 219° (decomp.). Analytical sample was obtained by recrystallization from MeOH as needles, mp 225° (decomp.). Concentration of the mother liquor of the recrystallization gave a further crop of the hydrazone (0.30 g). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3340, 3230, 1640. NMR ($d_6$-DMSO) $\delta$ ppm: 1.89 and 1.93 (3H, singlets, *ca.* 1:2, $C\underline{H}_3CO$–), 2.40 (3H, singlet, $C\underline{H}_3$–$C_6H_4$–), 4.53 (1H, broad, –N–$C\underline{H}$–C=), 6.4 (4H, multiplet, HO–$C_6\underline{H}_4$–), 7.68 (4H, $A_2B_2$-pattern, $CH_3$–$C_6\underline{H}_4$–), 9.13 and 10.31 (broad singlets, NH and OH). *Anal.* Calcd. for $C_{20}H_{23}O_4N_3S$: C, 59.84; H, 5.78; N, 10.47. Found: C, 59.69; H, 5.83; N, 10.92.

**1-Acetyl-2-(*p*-hydroxyphenylmethyl)-3-pyrroline (17)**——A solution of 1.23 g of **16** and 0.4 g of KOH in 5 ml of diethylene glycol was kept at 130—135° for 1.5 hr. The cooled reaction mixture was neutralized

with dil. AcOH, diluted with 50 ml of $H_2O$, and extracted with two 20 ml portions of benzene. The extract was washed twice with dil. $NaHCO_3$ solution, dried, and evaporated to dryness *in vacuo*, leaving 203 mg of a crystalline mass which was recrystallized from MeOH to 77 mg of **17** as prisms, mp 167—173°. The aqueous layer left after the extraction was further extracted with three 15 ml portions of $CHCl_3$ and the extract was evaporated to give 400 mg of further crop of **17**, mp 169—173°. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3130, 1640. NMR ($d_6$-DMSO) $\delta$ ppm: 1.96 and 2.05 (singlets, *ca.* 4:1, $C\underline{H}_3CO$–), 2.87 (2H, multiplet, Ar–$C\underline{H}_2$–), 3.5—4.35 (2H, multiplet, –$\underset{|}{N}$–$C\underline{H}_2$–CH=), 4.77 (1H, multiplet, –$\underset{|}{N}$–$C\underline{H}$–CH=), 5.75 (2H, multiplet, –$C\underline{H}$=$C\underline{H}$–), 6.79 (4H, $A_2B_2$-pattern, aromatic). *Anal.* Calcd. for $C_{13}H_{15}O_2N$: C, 71.86; H, 6.96: N, 6.45. Found: C, 71.31; H, 6.98: N, 6.32.

Treatment of **17** with $CH_2N_2$ in ether gave **13** in a good yield.

**1-Benzoyl-2-(*p*-methoxyphenylmethyl)-3-pyrroline (18)**——To a cooled solution of 2.42 g of **2** in 20 ml of benzene suspended with 3 g of $NaHCO_3$ (solid) was added 2 g of benzoyl chloride with stirring and the mixture was allowed to stand overnight. The reaction mixture was poured into ice–water and extracted with benzene. The benzene layer was washed with aqueous pyridine solution to remove excess of the reagent and successively washed with dil. HCl and $H_2O$. After drying, the solvent was removed *in vacuo*, leaving 3.86 g of **18**. Yield was quantitative. Analytical sample was obtained as a colorless liquid by silica gel column chromatography. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 1643, 1620. NMR ($CDCl_3$) $\delta$ ppm: 3.12 (2H, multiplet, Ar–$C\underline{H}_2$–), 3.79 (3H, singlet, –$OC\underline{H}_3$), *ca.* 3.8 (2H, multiplet, –$\underset{|}{N}$–$C\underline{H}_2$–CH=), 5.28 (1H, multiplet, –$\underset{|}{N}$–$C\underline{H}$–CH=), 5.72 (2H, multiplet, -$C\underline{H}$=$C\underline{H}$–), 7.02 (4H, $A_2B_2$-pattern, aromatic), 7.47 (5H, broad singlet, –CO–$C_6\underline{H}_5$), *ca.* 2.5 and 4.3 (weak broad signals). *Anal.* Calcd. for $C_{19}H_{19}O_2N$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.50; H, 6.57; N, 4.64.

**2α-(*p*-Methoxyphenylmethyl)-1-benzoyl-3β,4β- (25) and -3α,4α-epoxypyrrolidine (19)**——i) Epoxidation of **18** with Pertrifluoroacetic Acid at a Low Temperature: A pertrifluoroacetic acid solution, prepared from 1.8 ml (0.016 mole of trifluoroacetic anhydride and 0.3 ml of 90% $H_2O_2$ (0.013 mole) in dichloromethane, was added dropwise to an ice–cold solution of 1.40 g (0.0048 mole) of **18** in 12 ml of dichloromethane suspended with 6 g of $Na_2HPO_4$ with vigorous stirring during 30 min. The mixture was stirred for 1 hr with cooling and further for 1 hr at room temperature. The mixture was diluted with 25 ml of $H_2O$ and stirred for 1 hr to decompose the excess reagent. The organic layer was collected and the aqueous layer was washed with $CHCl_3$. The combined organic layer and washings was washed with $H_2O$, dried, and evaporated to dryness *in vacuo*, leaving 1.58 g of a syrup, which was chromatographed on 25 g of silica gel. Removal of the solvent from fractions eluted with benzene containing gradient amount of ether (2—5%, v/v) (200 ml) gave 140 mg of unchanged **18**. Fractions eluted with 5—10% (v/v) ether–benzene was evaporated to give 686 mg of crude **25** as a syrup which crystallized on standing. The crystals were collected and recrystallized from MeOH–ether to 521 mg of pure **25** as needles, mp 109—112° (40% yield based on unrecovered **18**.). IR $\nu_{max}^{Nujol}$: 1631 $cm^{-1}$. *Anal.* Calcd. for $C_{19}H_{19}O_3N$: C, 73.76; H, 6.19; N, 4.53. Found: C, 73.71; H, 6.23; N, 4.61.

ii) Epoxidation of **18** with Pertrifluoroacetic Acid at an Elevated Temperature: A pertrifluoroacetic acid solution, prepared from 3.2 ml (0.028 mole) of trifluoroacetic anhydride and 0.5 ml (0.022 mole) of 90% $H_2O_2$ in 3.1 ml of dichloromethane, was added dropwise to a solution of 3.86 g (0.013 mole) of **18** in 15 ml of dichloromethane suspended with 6 g of $Na_2CO_3$, with vigorous refluxing during 1 hr. After the addition was completed, the mixture was refluxed further for 30 min. The solid was collected and washed with dichloromethane. The combined filtrate and washings was evaporated to dryness *in vacuo*, leaving 3.63 g of a yellow syrup, which was chromatographed on 40 g of silica gel. Fractions eluted with 5% (v/v) ether–benzene (200 ml) were evaporated to give 994 mg of the unchanged material, fractions further eluted with 150 ml of 5% (v/v) ether–benzene gave 331 mg of a mixture of the crude α-epoxide (**19**) and the unchanged material and fractions eluted with 50 ml of ether–benzene (1:4, v/v) gave 177 mg of a mixture of **19** and **25** which partly crystallized on standing, giving 34 mg of the crystalline **25**. The remaining syrup and the afore–mentioned crude **19** were combined and chromatographed again on 8 g of silica gel. Elution with 3—5% (v/v) ether–benzene (40 ml) and evaporation of the solvent yielded 146 mg of further recovery of the unchanged material. The combined recovery was 1.14 g (30%). Fractions eluted with 5% (v/v) ether–benzene (120 ml) was evaporated to give 242 mg (9% based on the unrecovered material) of **19** as a colorless syrup. IR $\nu_{max}^{liq.}$ 1640 $cm^{-1}$. The sample of **19** did not give a satisfactory result on elementary analysis.

The column, after elution of the α-epoxide (**19**) fractions, was eluted with 500 ml of ether–benzene (1:4, v/v) and evaporation of the fractions yielded 1.59 g of crude **25** which crystallized on standing. The crystals were collected and washed with ether to give 1.361 g of **25**. Total yield of **25** was 1.395 g (49% based on the unrecovered material).

**1-Benzoyl-2α-(*p*-methoxyphenylmethyl)-3β-hydroxy-4α-acetoxy- (26), and -3β,4α-diacetoxypyrrolidine (28) (Acetolysis of the β-Epoxide (25))**——A solution of 714 mg of **25** and 110 mg of AcONa in 7 ml of AcOH was refluxed for 8 hr. The cooled mixture was poured into $H_2O$ and extracted with $CHCl_3$. The extract was successively washed with dil. $NaHCO_3$ and $H_2O$, dried, and evaporated *in vacuo*, yielding 911 mg of a syrup which was chromatographed on 18 g of silica gel. Fractions eluted with 10% (v/v) ether–benzene (150 ml) was evaporated to give 369 mg of a mixture of **28** and the unchanged **25**. Recrystallization of this

mixture from benzene–ether yielded 172 mg (20%) of the unchanged **25**, mp 106—108°, and the residual solution was concentrated to give 190 mg (19%) of **28** as a syrup. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 1749, 1642.

Further elution with ether–benzene (1:1, v/v, 150 ml) and removal of the solvent gave 395 mg (45%) of **26** as a colorless syrup. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3370, 1749, 1614. The samples of **26** and **28** did not give a satisfactory result on elementary analysis.

Treatment of **25** with AcOH for 15 hr gave **26** and **28** in 43% and 44% yield, respectively.

**2α-(*p*-Methoxyphenylmethyl)-3β,4α-dihydroxypyrrolidine** (**29**)——A solution of 171 mg of **28** and 0.5 g of KOH in 2 ml of 50% aqueous MeOH was refluxed for 15 hr. Treatment in the usual manner gave 52 mg of a syrup which crystallized on standing. Recrystallization from AcOEt–EtOH gave 29 mg of **29** as leaflets, mp 130—131.5°. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3390, 3290, 2700, 2570. *Anal.* Calcd. for $C_{12}H_{17}O_3N$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.43; H, 7.60; N, 6.38.

A solution of 175 mg of **28** in 8 ml of ether was treated with 80 mg of $LiAlH_4$ for 1 hr at room temperature and the product (135 mg) was hydrogenated over 10% Pd–C (50 mg) in 4 ml of EtOH for 1 hr. Treatment in the usual manner gave 89 mg of a syrup which was recrystallized from AcOEt–EtOH to 40 mg of **29** as leaflets, mp 127—129°.

The monoacetate (**26**) was also treated with a base as described above, giving **29** in a fair yield.

**Conversion of 26 into the α-Epoxide** (**19**)——**26** (150 mg) was dissolved in 2 ml of pyridine and 85 mg of MsCl was added with stirring and cooling. The mixture was allowed to stand overnight at room temperature, poured into an ice–water, and extracted with benzene. The extract was successively washed with dil. HCl, dil. $NaHCO_3$ solution, and $H_2O$, dried, and evaporated *in vacuo*, leaving 188 mg of crude **30** as a syrup. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 1751, 1643, 1180.

Without further purification, the crude **30** was dissolved in 10% MeOH solution of KOH and the mixture was stirred at room temperature for 10—15 min. The mixture was diluted with $H_2O$ and extracted with $CHCl_3$. The extract was washed with $H_2O$, dried, and evaporated *in vacuo*, giving 106 mg of the α-epoxide (**19**) as a syrup which was identified with the sample prepared before by means of thin-layer chromatography and IR spectrometry.

**Attempted Conversion of the β-Epoxide** (**25**) **into Anisomycin Analog** (**25→31→32→33**)——To a solution of 6 ml of benzyl alcohol containing 1% sodium benzyloxide was added 500 mg of **25** and the resulting mixture was kept at 85° for 15 hr. The cooled mixture was diluted with $H_2O$ and extracted with $CHCl_3$. After drying, the solvent was evaporated to dryness *in vacuo*, leaving 691 mg of a red syrup, which was chromatographed on 15 g of silica gel. After removal of benzyl alcohol (66 mg) by elution with 5% ether–benzene (60 ml), the column was eluted with benzene–ether (7:3, v/v, 100 ml) and evaporation of the solvent gave 152 mg of the unchanged **25** which was recrystallized to 133 mg of pure **25**. Further elution with benzene–ether (1:1, v/v, 300 ml) and evaporation of the solvent gave 381 mg of **31** as a syrup whose thin-layer chromatogram revealed one spot. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3360, 1612.

The crude **31** thus obtained was dissolved in 15 ml of dry ether and 0.1 g of $LiAlH_4$ was added. The solution was stirred for 1 hr at room temperature and treatment in the usual manner yielded 331 mg of a syrup which was acetylated with $Ac_2O$–pyridine, affording 334 mg of **32** as a syrup. **32** also revealed one spot on thin-layer chromatogram. IR $\nu_{max}^{liq.}$: 1742 $cm^{-1}$. **32** thus obtained (312 mg) was hydrogenated over 10% Pd–C (70 mg) in 5 ml of MeOH for 2 hr. After removal of the catalyst, the mixture was evaporated *in vacuo*, yielding 250 mg of **33** as a syrup. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3320 (weak, NH), 1741 (no hydroxyl absorption).

**Attempted Conversion of the α-Epoxide** (**19**) **into Anisomycin** (**19→20 and 34→21 and 35→22 and 36→37 and 38**)——To a solution of benzyl alcohol (3 ml) containing 1% sodium benzyloxide was added 250 mg of **19** and the mixture was kept at 95° for 15 hr with stirring. The mixture was diluted with $H_2O$ and extracted with benzene. The benzene extract was dried and evaporated to dryness *in vacuo*, affording 270 mg of a syrup which was chromatographed on 7 g of silica gel. After elution with 80 ml of 10% (v/v) benzene–ether, the column was eluted with benzene–ether (4:1, v/v). The fast running fraction (60 ml) was evaporated to give 112 mg of one main component (IR $\nu_{max}^{liq.}$: 3360, 1614) and the slow running fraction (80 ml) to give 105 mg of the other (IR $\nu_{max}^{liq.}$: 3350, 1611.). These products were pure on thin-layer chromatogram, but did not give a satisfactory data on elementary analysis. Either of them (**20** or **34**) was treated with $LiAlH_4$, successively acetylated, and hydrogenolysed over Pd–C. However, infrared spectra of the final product (**37** or **38**) thus obtained showed that either of the O-benzyl group at 3 or 4 position did not detached as in the case described above for **33**.

**2-(*p*-Methoxyphenylmethyl)-1-benzyloxycarbonyl-3-pyrroline** (**39**)——To a cooled solution of 9.9 g of **2** in 100 ml of benzene suspended with 10 g of $NaHCO_3$ (solid) was added 10.2 g of benzyloxycarbonyl chloride and the mixture was stirred for 2 hr at room temperature. The reaction mixture was poured into ice–water and extracted with benzene. The extract was dried and evaporated to dryness *in vacuo*, leaving 17.34 g of **39** as a syrup which was homogeneous on thin-layer chromatography. Analytical sample was obtained by silica gel chromatography. Attempted purification by distillation was not successful. IR $\nu_{max}^{liq.}$: 1708 $cm^{-1}$. NMR ($CDCl_3$) $\delta$ ppm: 3.13 (2H, multiplet, Ar–C<u>H</u>$_2$–), 3.79 (3H, singlet, –OC<u>H</u>$_3$), *ca.* 3.8 (2H, multiplet, –N(–C<u>H</u>$_2$–)–CH=), 5.28 (1H, multiplet, –N(–C<u>H</u>–)–CH=), 5.73 (2H, multiplet, –C<u>H</u>=C<u>H</u>–), 7.03

(4H, $A_2B_2$-pattern, aromatic), 7.47 (5H, broad singlet, $C_6\underline{H}_5CO$–), and weak broad signal at 2.5 and 4.3. *Anal.* Calcd. for $C_{20}H_{21}O_3N$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.14; H, 6.66; N, 4.55.

**2α-(*p*-Methoxyphenylmethyl)-1-benzyloxycarbonyl-3α,4α- (40) and -3β,4β-epoxypyrrolidine (41)**——To a boiling and stirred solution of 16.2 g (0.05 mole) of **39** in 60 ml of dichloromethane suspended with 23 g of $Na_2CO_3$ was added dropwise during 30 min pertrifluoroacetic acid prepared from 12 ml (0.10 mole) of trifluoroacetic anhydride and 2 ml (0.088 mole) of 90% $H_2O_2$ in 13 ml of dichloromethane. The mixture was refluxed further for 1 hr with stirring. When cooled, the mixture was filtered and the collected solid was washed with dichloromethane. The combined filtrate and washings was dried and evaporated to dryness *in vacuo*, leaving 13.36 g of a red syrup, which was chromatographed on 200 g of silica gel. Fractions eluted with benzene was evaporated to give 4.18 g (26%) of unchanged **39** and fractions eluted with benzene–ether (10:1, v/v) gave 6.33 g of a mixture of the α-epoxide (**40**) and the β-epoxide (**41**), along with an unidentified product. The mixture was repeatedly chromatographed on silica gel. The fast running part afforded the α-epoxide (**40**) as a syrup and the slow running part the β-epoxide (**41**) also as a syrup.

Each component was characterized by thin-layer chromatography. Thus, 0.9 g (7% based on the unrecovered **39**) of **40** and 3.5 g (35%) of **41** were obtained. **40** solidified on a long standing, mp 57—61°. No suitable solvent for recrystallization was found. An amorphous substance, mp 58.5—61°, deposited very slowly from EtOH. *Anal.* Calcd. for $C_{20}H_{21}O_4N$: C, 70.78; H, 6.24; N, 4.13. Found (for **40**): C, 70.61; H, 6.32; N, 4.23. Found (for **41**): C, 70.44; H, 6.24; N, 4.19.

**2α-(*p*-Methoxyphenylmethyl)-3β,4β-epoxypyrrolidine(42)**——A slow stream of $H_2$ was passed through a stirred solution of 356 mg of **41** in 2 ml of EtOH in the presence of 50 mg of 10% Pd–C for 1 hr. After removal of the catalyst, the mixture was evaporated *in vacuo*, giving 205 mg of crude **42**, mp 81.5—85°. The analytical sample, mp 83.5—85.5°, was purified by sublimation at 130—140° under a reduced pressure (1 mmHg). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 1613, 1511, 1462, 1250, 1180, 1030, 894, 840. *Anal.* Calcd. for $C_{12}H_{15}O_2N$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.48; H, 7.10; N, 6.88.

Benzoylation of 36 mg of **42** with benzoyl chloride in benzene in the presence of $Na_2CO_3$ afforded 33 mg of **25**, mp 109—112°.

**2α-(*p*-Methoxyphenylmethyl)-1-benzyloxycarbonyl-3β-hydroxy-4α-acetoxy- (43) and - 3β,4α-diacetoxypyrrolidine (44) (Acetolysis of the β-Epoxide (41))**——A solution of 1.2 g of **41** and 0.15 g of AcONa in 5 ml of AcOH was refluxed for 6.5 hr. The reaction mixture was concentrated *in vacuo*, diluted with $H_2O$, and extracted with ether. The extract was washed with dil. $NaHCO_3$ solution and $H_2O$, dried, and evaporated *in vacuo* leaving 1.40 g of a brown syrup which exhibited 2 spots on thin-layer chromatogram. The syrup was chromatographed over 15 g of silica gel. Fractions eluted with ether–benzene (1:10, v/v) were evaporated to yield 559 mg of the unchanged material containing a diacetate (**44**) which was shown by IR spectrometry. Fraction eluted with benzene–ether (1:1, v/v) were evaporated to yield 795 mg (56%) of **43** as crystals of mp 101—111°, which was recrystallized from EtOH to 656 mg of prisms, mp 112.5—113°. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3380, 1745, 1670. NMR ($CDCl_3$) δ ppm: 2.11 (3H, singlet, $C\underline{H}_3COO$–), 3.75 (3H, singlet, $C\underline{H}_3O$–), 5.0 (1H, multiplet, AcO–$C\underline{H}$–), 5.14 (2H, singlet, –$COOC\underline{H}_2Ph$), 6.95 (4H, $A_2B_2$-pattern, aromatic), 7.37 (5H, singlet, phenyl). *Anal.* Calcd. for $C_{22}H_{25}O_6N$: C, 66.15; H, 6.31; N, 3.51. Found: C, 66.42; H, 6.32; N, 3.31.

Similarly, **41** was treated with AcOH for 15 hr and the reaction product was chromatographed on silica gel. Fractions eluted with ether–benzene (1:10, v/v) were evaporated to give the diacetate (**44**) as a syrup in 41% yield. along with a small amount of the unchanged material. Analytically pure sample was not obtained. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 1750, 1710. NMR ($CDCl_3$) δ ppm:1.94 (3H, singlet, $C\underline{H}_3COO$–), 2.10 (3H, singlet, $C\underline{H}_3COO$–), 3.76 (3H, singlet, $C\underline{H}_3O$–), 5.15 (2H, multiplet, AcO–$C\underline{H}$–), 5.17 (2H, singlet, –$COOC\underline{H}_2Ph$), 7.0 (4H, multiplet, aromatic), 7.37 (5H, singlet, phenyl).

The NMR spectrum of **44** showed that the presence of the isomeric 3α,4β-diacetoxypyrrolidine (**51**) was less than 5%. Moreover, fractions eluted with benzene–ether (1:1, v/v) were evaporated to yield **43** in 51% yield.

**Conversion of 43 into the α-Epoxide (40)**——To a solution of 960 mg of the crude **43** in 7 ml of pyridine was added 400 mg of mesyl chloride with cooling and stirring, and the mixture was allowed to stand overnight. The mixture was poured into ice–water and extracted with ether. The extract was successively washed with dil. HCl, dil. $NaHCO_3$, and $H_2O$, dried, and evaporated *in vacuo*, leaving 1.15 g of a pale-yellow syrup which was dissolved in 10 ml of EtOH. To the resulting solution was added 10% KOH–EtOH solution (5 ml). After stirring for 15 min at room temperature, the mixture was diluted with $H_2O$ and extracted with benzene. The extract was dried and evaporated *in vacuo*, giving 734 mg (89%) of α-epoxide (**40**) as a colorless syrup, which was identified with the sample obtained before by IR and thin-layer chromatography.

**2α-(*p*-Methoxyphenylmethyl)-1-benzyloxycarbonyl-3β,4α-dihydroxypyrrolidine (46)**——The crude diacetate (**44**) obtained above (1.13 g) was dissolved in a mixture of 12 ml of EtOH and 10 ml of $H_2O$ containing 2 g of KOH and the mixture was refluxed for 3 hr. The reaction mixture was concentrated to half the volume and extracted with $CHCl_3$. The extract was washed with dil. HCl and $H_2O$, dried, and evaporated *in vacuo*, leaving 727 mg of crude **46** as a syrup. The remaining aqueous layer was basified with conc. KOH solution and extracted several times with $CHCl_3$. The extract was dried and evaporated to give 74 mg of crystals

which were recrystallized from EtOH–AcOEt to 36 mg of **29** as leaflets, mp 126.5—129°, which was identified with the aforementioned sample by mixed mp and IR spectrometry.

The crude **46** was chromatographed on 2 g of silica gel. After washing with benzene–ether (2:1, v/v) for removal of impurity, elution with 1% MeOH–benzene and removal of the solvent yielded 390 mg of crystals, mp 101—103.5°, which were recrystallized from ether–benzene to prisms, mp 101—104°. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3430, 1688. *Anal.* Calcd. for $C_{20}H_{23}O_5N$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.00; H, 6.57; N, 3.93.

**46** (112 mg) was hydrogenated over 30 mg of 10% Pd–C in 1.5 ml of EtOH in a slow stream of $H_2$. After filtration of the catalyst, the solvent was evaporated to give 65 mg of crystals, mp 123—127°, which were recrystallized from AcOEt–EtOH to give 50 mg of **29**, mp 129—131°, which was also identified with the sample obtained before.

**4α-Acetoxy-2α-(*p*-methoxyphenylmethyl)-3β-hydroxypyrrolidine (47)**——A slow stream of $H_2$ was passed through a solution of 115 mg of **43** in 1.5 ml of EtOH in the presence of 20 mg of 10% Pd–C during 3 hr with stirring. After removal of the catalyst, the mixture was evaporated *in vacuo*, giving a crystalline mass (64 mg), mp 142—144°, which was recrystallized from EtOH to 49 mg of **47** as leaflets, mp 147—148.5°. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3300, —3050, —2700, 1733, 1514, 1467, 1377, 1254, 1031, 840. NMR ($d_6$–DMSO) $\delta$ ppm: 2.01 (3H, singlet, $C\underline{H}_3CO$–), *ca.* 2.4—3.4 (7H, multiplet), *ca.* 3.7 (1H, multiplet, –C<u>H</u>–OH), 3.72 (3H, singlet, $-OC\underline{H}_3$), 4.78 (1H, multiplet, –C<u>H</u>–OAc), 7.03 (4H, $A_2B_2$-pattern, aromatic). *Anal.* Calcd. for $C_{14}H_{19}O_4N$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.21; H, 7.30; N, 5.48.

***dl*-Anisomycin (1) and 3β-Acetoxy-2α-(*p*-methoxyphenylmethyl)-4α-hydroxypyrrolidine (52)**——The α-epoxide (**40**) (2.35 g) and sodium trifluoroacetate (0.3 g) were dissolved in 9 ml of trifluoroacetic acid and the mixture was allowed to stand for 2 hr at room temperature. Evaporation of trifluoroacetic acid *in vacuo* at room temperature left a syrup which was diluted with 10 ml of dry benzene. After the solid (sodium trifluoroacetate) was filtered off, the filtrate was evaporated to dryness *in vacuo* at room temperature and finally at 70°, leaving 3.40 g of a mixture of **48** and **49** as a red syrup. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3400, 1787, 1676.

The syrup thereby obtained was dissolved in 20 ml of pyridine and 2.5 g of $Ac_2O$ was added. After the mixture was stood for 1 hr at room temperature, it was poured into ice–water and extracted with benzene. The extract was washed with dil. HCl and $H_2O$, dried, and evaporated *in vacuo*, yielding 2.63 g of a mixture of **43** and **50** as a red syrup, which were chromatographed over 35 g of silica gel. Elution with 10% (v/v) ether–benzene (150 ml) and removal of the solvent gave 0.58 g of a diacetate mixture of **44** and **51**. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 1750, 1710. The NMR spectrum indicated that the relative ratio of **44** (characteristic acetyl bands: 1.94 and 2.10 ppm) and **51** (characteristic acetyl bands: 1.99 and 2.03 ppm) was 47:53. Removal of the solvent from the fraction eluted with 20% (v/v) ether–benzene (800 ml) gave 1.67 g of a mixture of **43** and **50**. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3430, 1748, 1707, 1690 (shoulder). Further elution with 5% (v/v) MeOH–$CHCl_3$ afforded a complex mixture containing some dihydroxyl derivatives.

The mixture of monoacetates (**43** and **50**) was dissolved in 10 ml of EtOH and the solution was hydrogenated over 10% Pd–C (0.3 g) in a slow stream of $H_2$ for 1.5 hr. After filtration, the mixture was evaporated to dryness *in vacuo*, leaving 978 mg of a crystalline syrup, which was recrystallized from benzene–hexane (*ca.* 5:1) to 315 mg (17.2% from **40**) of **47**, mp 142—146°. Further recrystallization from EtOH gave leaflets, mp 147—148.5°, which was identical with the sample obtained before by mixed mp and IR spectrometry.

The mother liquor of the recrystallization of **47** was evaporated and the residue was dissolved in benzene–hexane (*ca.* 5:1). Crystals formed by standing in a refrigerator overnight was collected and recrystallized from AcOEt–hexane (*ca.*5:1) yielding 155 mg of **1** as needles, mp 118—121°. The mother liquor was concentrated again and chromatographed over 18 g of silica gel. Elution with 5% (v/v) MeOH–$CHCl_3$ and removal of the solvent gave 262 mg of a crystalline syrup which was recrystallized three times from AcOEt–hexane, affording a second crop of **1** (30 mg), mp 117—120°. The IR ($CHCl_3$) and NMR spectra ($CDCl_3$) of **1** were completely identical with those of natural anisomycin. Repeated recrystallization of **1** from benzene–hexane or AcOEt–hexane gave polymorphic crystals which showed a higher, but not-sharp melting point (mp 121—126°, mp 127—128.5°, mp 130.5—140°). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3300, 3170, 2750, 1739, 1516, 1464, 1381, 1250, 1038, 966, 895. NMR ($CDCl_3+D_2O$) $\delta$ ppm:2.12 (3H,singlet,$C\underline{H}_3CO$–), 3.76 (3H, singlet, $-OC\underline{H}_3$), 4.14 (1H, ddd, $J$=6, 5 and 1 cps, –C<u>H</u>–OH), 4.71 (1H, dd, $J$=4.7, and 1 cps, –C<u>H</u>–OAc). *Anal.* Calcd. for $C_{14}H_{19}O_4N$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.10; H, 7.18; N, 5.27.

The mother liquor left after recrystallization of **1** was concentrated *in vacuo* and the residual syrup was chromatographed on 9 g of silica gel. After elution with 1% (v/v) MeOH–$CHCl_3$ (60 ml) for removal of some impurities, elution with the same solvent (90 ml) and evaporation of the solvent yielded 55 mg of crystals which were recrystallized from AcOEt–hexane to 24 mg of **52** as needles, mp 100.5—101.5°. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3380, 2670, 1735, 1516, 1466, 1380, 1250, 1029, 921. *Anal.* Calcd. for $C_{14}H_{19}O_4N$: C, 63.38: H, 7.22; N, 5.28. Found: C, 63.40; H, 7.26; N, 5.16.

Further elution with the same solvent (90 ml) yielded 33 mg of a mixture of **1** and **52**. Elution with 5% (v/v) MeOH–$CHCl_3$ (200 ml) and removal of the solvent gave 119 mg of a syrup which partly crystallized

on standing. Recrystallization from benzene–hexane gave the third crop (23 mg) of **1**. Total yield of **1** from **40** was 11.4%.

**4β-Acetoxy-2α-(*p*-methoxyphenylmethyl)-3α-hydroxypyrrolidine (53)**——A solution of 298 mg of the α-epoxide (**40**) in 5 ml of EtOH was hydrogenated over 50 mg of 10% Pd–C for 30 min in a slow stream of $H_2$. After the catalyst was filtered off, the filtrate was evaporated to dryness *in vacuo*, leaving 167 mg of crude 3α,4α-epoxypyrrolidine (**23**) as a semi-solid, which showed no clear melting point (the end point at 45°). IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3320, 1614, 1513, 1247, 1178, 1033.

A solution of 106 mg of the epoxide (**23**) thereby obtained in 1.5 ml of AcOH was refluxed for 2.5 hr. The mixture was diluted with $H_2O$ and washed with benzene. The aqueous layer was basified with $Na_2CO_3$ solution and extracted three times with $CHCl_3$. Evaporation of the solvent from the extract left 95 mg of a crystalline mass which was recrystallized from AcOEt containing a small amount of EtOH, yielding 46 mg (36%) of **53** as needles, mp 144—146°. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3280, 2710, 1730, 1512, 1462, 1372, 1249, 1034, 962, 909. *Anal.* Calcd. for $C_{14}H_{19}O_4N$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.36; H, 7.10; N, 5.25.

**Acetolysis of the α-Epoxide (40)**——A solution of 104 mg of **40** and 35 mg of AcONa in 1 ml of AcOH was refluxed for 2 hr. The cooled mixture was poured into $H_2O$ and extracted with benzene. The extract was washed with dil. $NaHCO_3$ solution and $H_2O$, dried, and evaporated *in vacuo*, leaving 129 mg of a syrup. The syrup thus obtained was acetylated with $Ac_2O$ in pyridine and treatment in the usual manner gave 130 mg of a mixture of **44** and **51**. *Anal.* Calcd. for $C_{24}H_{27}O_7N$: C, 65.29; H, 6.16; N, 3.17. Found: C, 65.32; H, 6.17; N, 3.03.

The NMR spectrum of the mixture of **44** and **51** showed that their relative ratio was 53:47.

**2α-(*p*-Methoxyphenylmethyl)-3β-hydroxypyrrolidine (54)**——To a stirred solution of 344 mg of **42** in 20 ml of ether was added 150 mg of $LiAlH_4$ and the mixture was stirred for 2 hr at room temperature. After careful addition of AcOEt and $H_2O$, the mixture was filtered and the filtrate was extracted three times with $CHCl_3$. The extract was dried and evaporated *in vacuo* to give 341 mg of a crystalline residue which was recrystallized from AcOEt, to 248 mg (71%) of **54** as needles, mp 110—111°. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3300, 3050, 2720, 1513, 1464, 1248, 1180, 1087, 1039, 823. NMR ($CDCl_3$+$D_2O$) δppm: 3.79 (3H, singlet, $-OC\underline{H}_3$), 3.98 (1H, ddd, $J$=6.5, 4, and 4 cps, $-C\underline{H}OH$), 7.01 (4H, $A_2B_2$-pattern aromatic). *Anal.* Calcd. for $C_{12}H_{17}O_2N$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.35; H, 8.34; N, 7.13.

A mixture of 102 mg of **54**, 0.3 ml of $Ac_2O$, and 2 ml of MeOH was allowed to stand overnight. Treatment in the usual manner gave 148 mg of the N-acetate of **54** as a syrup (IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3380, 1620) which was dissolved in 1 ml of AcOH and 0.1 g of $CrO_3$ was added with stirring at room temperature. After standing for 10 hr, the mixture was diluted with $H_2O$ and extracted with $CHCl_3$. The extract was washed with dil. $NaHCO_3$, dried, and evaporated *in vacuo*, leaving a syrup (30 mg). IR $\nu_{max}^{liq.}$ $cm^{-1}$: 1761 (five-membered ketone). The syrup formed a tosylhydrazone (**11**), mp 210—215° (decomp.), which was identified with the sample prepared before.

**2α-(*p*-Methoxyphenylmethyl)-3α-hydroxypyrrolidine (56)**——The pyrrolidine-3-one (**10**) (2.35 g) purified by chromatography was dissolved in 25 ml of MeOH and 350 mg of $NaBH_4$ was added in small portions with stirring. The mixture was stood at room temperature for 20 min and the mixture was concentrated *in vacuo* to one-quarter the original volume. The concentrate was diluted with $H_2O$ and extracted three times with $CHCl_3$. Evaporation of the solvent left 2.35 g of **55** as a syrup. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3350, 1615. *Anal.* Calcd. for $C_{14}H_{19}O_3N$: C, 67.44; H, 7.68; N, 5.62. Found: C, 67.68; H, 7.63; N, 5.17.

A solution of 2.35 g of **55** thus obtained and 2.5 g of KOH in 12 ml of ethylene glycol was kept at 160—170° for 6 hr and the cooled solution was diluted with 10 ml of $H_2O$. The mixture was extracted with 20 ml of $CHCl_3$ and the extract was shaken with 5 ml of 6 N HCl. The aqueous layer was basified with dil. NaOH solution, and extracted three times with $CHCl_3$.

Evaporation of the solvent left 1.55 g of a crystalline residue, which was recrystallized from AcOEt-ether to 1.20 g (62% from **10**) of **56** as fine needles, mp 113—114.5°. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3270, 2700, 1512, 1460, 1248, 1034, 905. NMR ($CDCl_3$) δ ppm: 2.58, 2.88 (2H, singlets, N$\underline{H}$ and O$\underline{H}$), 3.79 (3H, singlet, $-OC\underline{H}_3$), 4.06 (1H, multiplet, $-C\underline{H}$–OH), 7.07 (4H, $A_2B_2$-pattern, aromatic). *Anal.* Calcd. for $C_{12}H_{17}O_2N$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.75; H, 8.34; N, 6.73.

The hydrochloride was obtained as fine needles, mp 229—235° (decomp.). *Anal.* Calcd. for $C_{12}H_{18}O_2NCl$: C, 59.13; H, 7.44; N, 5.75. Found: C, 59.03; H, 7.52; N, 5.85.

**3α-Acetoxy-2α-(*p*-methoxyphenylmethyl)-pyrrolidine (Deoxyanisomycin) (57)**——To a cooled solution of 1.20 g of **56** in a mixture of 20 ml of benzene and 5 ml of $CHCl_3$ with suspended $Na_2CO_3$ (1 g) was added a solution of 1.1 g of benzyloxycarbonyl chloride in 10 ml of benzene with stirring during 30 min. The mixture was stirred for 2 hr at room temperature and diluted with $H_2O$. The organic layer was successively washed with $H_2O$, dil. HCl solution, and $H_2O$, dried, and evaporated *in vacuo*, leaving 1.95 g of a yellow syrup, which was homogeneous by thin-layer chromatography. Analytical sample was obtained by chromatography on silica gel. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3430, 1675. *Anal.* Calcd. for $C_{20}H_{23}O_4N$: C, 70.36; H, 6.79; N, 4.10. Found: C, 70.22; H, 6.87; N, 4.07.

The N-benzyloxycarbonyl derivative (1.14 g) thus obtained was dissolved in 10 ml of pyridine and 1.5 g of $Ac_2O$ was added. The mixture was warmed on a steam bath for 1.5 hr and poured into $H_2O$. The mixture

was extracted with benzene, the extract was washed with dil. HCl and $H_2O$, and evaporated to give 1.26 g of a colorless syrup. IR $\nu_{max}^{liq.}$ $cm^{-1}$: 1742, 1704. *Anal.* Calcd. for $C_{22}H_{25}O_5N$: C, 68.91; H, 6.57; N, 3.65. Found: C, 69.06; H, 6.56; N, 3.62.

The acetate (920 mg) thereby obtained was dissolved in 10 ml of EtOH and hydrogenated over 10% Pd–C (0.1 g) for 1.5 hr. After filtration of the catalyst, evaporation of the solvent gave 588 mg of **57** as a pale yellow syrup, bp 160—165° (0.1 mmHg, bath temp.). IR $\nu_{max}^{liq.}$ $cm^{-1}$: 3340, 1738, 1513, 1245, 1132. NMR ($CDCl_3$) $\delta$ ppm: 2.09 (3H, singlet, $C\underline{H}_3COO$–), 2.30 (1H, broad singlet, –$N\underline{H}$), 3.77 (3H, singlet, $C\underline{H}_3O$–), 5.15 (1H, multiplet, –$C\underline{H}$–OAc), 6.97 (4H, $A_2B_2$-pattern, aromatic). *Anal.* Calcd. for $C_{14}H_{19}O_3N$: C, 67.44; H, 7.68; N, 5.62. Found: C, 66.71; 7.70; N, 5.73.

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