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CAPREOMYCIDINE AND 3-GUANIDINOPROLINE FROM VIOMYCIDINE

SYNTHESIS OF *CIS* AND *TRANS* 3-AMINOPROLINES

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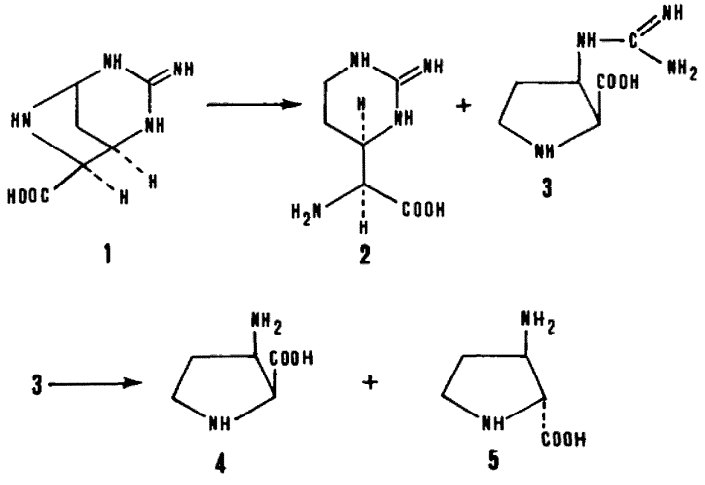
Abstract—*cis*-3-Guanidino-L-proline and capreomycidine have been obtained by catalytic hydrogenation of viomycidine. The configuration of the 3-aminoproline isolated in the alkaline hydrolysis of guanidinoproline has been determined by means of ORD data and DAO oxidation. Assignement of *trans* geometry to the 3-aminoproline is based on the synthesis of both the *cis* and *trans* 3-aminoprolines.

IN A previous paper[1] we noted that catalytic hydrogenation of viomycidine (**1**) yields two main ninhydrin-positive products, one of which had been isolated and identified as a (+)-3-guanidinoproline (**3**). The alkaline hydrolysis of this compound gave a 3-aminoproline the geometry of which had not been determined, but only its D configuration at C-2 by means of ORD data.



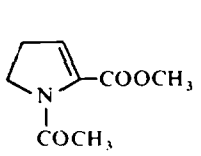
In the present paper we wish to explain the stereochemical relations between the (+)-3-guanidinoproline and the 3-amino-D-proline obtained and the nature of the second compound isolated in the hydrogenation of viomycidine.

In view of the two possibilities of opening the N—C—N system of viomycidine, this second compound proved to be capreomycidine (**2**) recognized by its physical constants, mainly the NMR spectrum.[3] This finding allows a direct comparison of the asymmetry

centres common to the two molecules* and confirms the absolute configuration[5] of capreomycidine.

The absolute configuration of the (+)-3-guanidinoproline is similarly defined: L configuration at C-2 and *cis* geometry. Simple hydrolysis of the guanidino-group should lead to *cis*-3-amino-L-proline, while ORD data of the material obtained by hydrolysis showed D configuration at C-2. Clearly epimerization occurred at this position during reaction to yield a mixture of *cis*-3-amino-L-proline and *trans*-3-amino-D-proline, in which the thermodynamically more stable *trans* isomer prevails and can easily be isolated by crystallization.

In order to check this hypothesis, we synthesized both the *cis* and *trans* isomers of 3-amino-D, L-proline by the methods reported. Only the *cis*-3-amino-D,L-proline had been prepared previously.[6]



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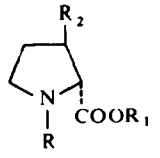

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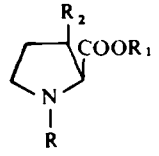


L-forms only shown

	R	R_1	R_2		R	R_1	R_2
6	Cbz	CH_3	DH	**7**	Cbz	CH_3	OH
8	Cbz	CH_3	OTos	**9**	Cbz	CH_3	OTos
11	Cbz	CH_3	N_3	**10**	Cbz	CH_3	N_3
12	H	H	NH_2	**13**	H	H	NH_2
15	$COCH_3$	CH_3	$C_6H_5CH_2NH$				
16	$COCH_3$	H	$C_6H_5CH_2NH$				
17	$COCH_3$	H	NH_2				

The first method involved the use of the *cis* and *trans* isomers of 3-hydroxy-N-carbobenzyloxy-D,L-proline methyl ester[7] as starting material. The *cis* isomer (**7**) was transformed into the corresponding O-tosyl derivative (**9**) and then into the *trans* azide (**11**) by displacement with azide ion with inversion at C-3. Catalytic hydrogenation followed by alkaline hydrolysis of the ester function gave trans-3-amino-D,L-proline (**12**).

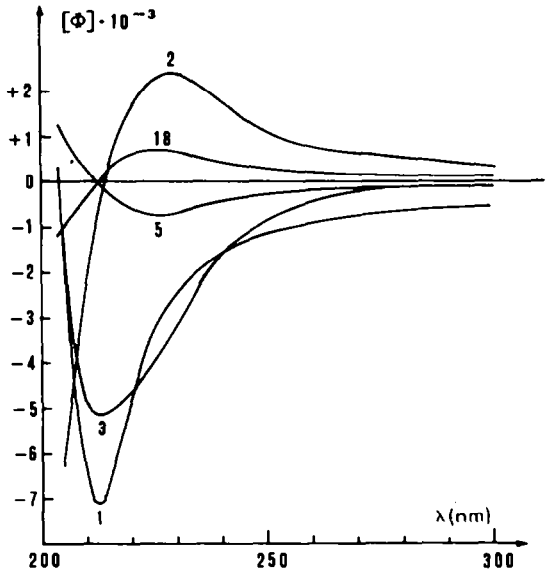
Synthesis of *cis*-3-amino-D,L-proline (**13**) paralleled that of the trans isomer. In this case the *cis* azide (**10**) contained traces of the *trans* isomer (**11**) and the starting tosylate (**8**).

The second method involved addition of benzylamine to methyl-1-acetyl-Δ^2-pirroline-2-carboxylate. Saponification of the ester function, hydrogenolysis of the benzylamino group and acid hydrolysis of the amido function gave *trans*-3-amino-D,L-proline.

The 3-aminoproline obtained by hydrolysis of the (+)-3-guanidinoproline proved to be identical with the *trans* isomer. Its configuration at C-2 was further confirmed by comparison with *trans*-3-amino-L-proline (**18**) prepared by DAO oxidation† of the racemic compound. The two products proved to be identical except for the optical properties, displaying ORD curves identical in shape and opposite in sign.

* The absolute configuration of viomycidine has been demonstrated by X ray analysis[4]

† We did not succeed in finding examples of DAO oxidation on β-aminoacids; experiments carried out in our laboratory on β-alanine showed that the β-amino group is not affected as in the case of trans-3-amino-L-proline.



ORD curves in 0·1 N HCl: capreomycidine (**2**); *trans*-3-amino-L-proline (**18**); *trans*-3-amino-D-proline (**5**); *cis*-3-guanidino-L-proline (**3**) and viomycidine (**1**).

EXPERIMENTAL

M.ps (Büchi oil bath apparatus) are uncorrected. IR spectra were recorded on a Perkin-Elmer 521 spectrophotometer. NMR spectra of all the compounds described were recorded on a Varian A-60 or Varian HA 100 instrument. In every case only absorption of expected chemical shift and area was observed. A detailed interpretation and an analysis of the fine structure will be reported in another paper. Rotations were determined on a Schmidt-Haensch polarimeter with 1 dm cell and ORD curves recorded on a Cary 60 instrument with 1 mm cell in 0·1N HCl. Identity of samples prepared by alternative routes was established by m.p., mixed m.p. and superposability of their IR and NMR spectra. Merck Silica Gel was employed for TLC and Davison Silica Gel 200–400 mesh for column chromatography.

Viomycidine (**I**). Viomycidine hydrochloride was isolated from the acid hydrolysate of viomycin by ion exchange resin chromatography: m.p. 207–208° dec; $[\alpha]_D^{25}$ −73° (*c*, 1·78 in H_2O); lit[8]: m.p. 200–208° dec; $[\alpha]_D$ −78° (*c*, 1·78 in H_2O). (Found: C, 34·71; H, 5·34; N, 27·10; Cl, 17·11. $C_6H_{11}N_4O_2Cl$ requires: C, 34·88; H, 5·37; N, 27·11; Cl, 17·16%).

Catalytic hydrogenation of viomycidine. A soln of **1** free base (500 mg) in AcOH (30 ml) was hydrogenated over 10% Pd/C (1g) at 50° and 3 atm. After 18 hr the catalyst was removed and the solvent evaporated *in vacuo*. The residue was acidified with 5 ml 1N HCl and the soln taken to dryness *in vacuo*. The glassy material was dissolved in 2 ml H_2O and crystallized by adding 20 ml EtOH and increasing amounts of ether. Recrystallization from H_2O—EtOH gave 140 mg of **3**, m.p. 245° dec; $[\alpha]_D^{25}$ +36·5° (*c*, 1·78 in H_2O). (Found: C, 34·30; H, 6·41; N, 26·66; Cl, 16·90. $C_6H_{13}N_4O_2Cl$ requires: C, 34·54; H, 6·28; N, 26·85; Cl, 16·99%).

The mother liquor of the first crystallization was taken to dryness *in vacuo*, the residue dissolved with water, the pH adjusted to 7 by addition of small amounts of Amberlite IR 4B resin and the resin removed by filtration. The soln was taken to dryness *in vacuo* and the oily material was crystallized once from MeOH and then from H_2O—EtOH to give 110 mg of **2**: m.p. 245° dec, $[\alpha]_D^{25}$ +25° (*c* 0·94 in 6N HCl); lit.[3]: m.p. 241° dec, $[\alpha]_D^{20}$ +35° (*c*, 0·88 in 6N HCl); NMR spectrum in D_2O identical with that reported.[3] (Found: C, 34·46; H, 6·36; N, 26·75. $C_6N_{13}N_4O_2Cl$ requires: C, 34·54; H, 6·28; N, 26·85%).

Hydrolysis of cis-3-*guanidino*-L-*proline* (**3**). A soln of 3-hydrochloride (160 mg) and Ba $(OH)_2 . 8H_2O$ (950 mg) in H_2O (6 ml) was refluxed under N_2 for 48 hr. The pH was adjusted to 7 with dil H_2SO_4 and $BaSo_4$ removed by centrifugation. The clear soln was passed through a column containing 5 ml Dowex 50 W × 4

resin (H^+ form), the column washed with water and the basic products recovered by eluting with 0·1M NH_4OH. Evaporation of the combined ninhydrin-positive fractions gave an oily residue which proved to be a mixture (about 1 : 3 *cis-trans* ratio) of **5** and **4** (TLC: 95% EtOH 25% NH_4OH 3 : 1; comparison with racemic synthetic samples). This material was acidified with HCl and crystallized by addition of EtOH to give 45 mg of **5** hydrochloride: m.p. 228° dec; $[\alpha]_D^{25}$ −20° (*c*, 1·3 in 0·1N HCl). (Found: C, 36·27; H, 16·67; N, 16·60. $C_5H_{11}H_2O_2Cl$ requires: C, 36·05; H, 6·66; N, 16·81%).

cis-3-*Amino*-D,L-*proline* (**13**). To an ice-cold soln of **6** (2·7 g) in dry pyridine (10 ml) a cold soln of tosyl chloride (4 g) in pyridine (10 ml) was added and the mixture allowed to stand for 4 days at room temp. The mixture was chilled in an ice-bath and 6 ml H_2O added. After 20 min the solvent was removed *in vacuo* and the residue dissolved with 200 ml EtOAc. The soln was washed with three 100 ml portions of 2N HCl, two 100 ml portions of saturated $NaHCO_3$ aq and three 100 ml portions of saturated salt soln, each fraction being re-extracted with a further portion of EtOAc. The combined EtOAc extracts were dried on Na_2SO_4 and evaporated *in vacuo* to give 4·17 g (98% yield) of **8** as a thick oily residue, showing one spot on TLC. A portion of this material was chromatographated on a column of silica gel: elution was effected with C_6H_6–$CHCl_3$ 1 : 1 mixture and a center fraction used for IR and NMR spectra. IR (liquid film), cm^{-1}: 1745 (ester C=O), 1705 (urethane C=O), 1592 and 1490 (aromatic ring), 1355 and 1200 (tosylate SO_2), 1170 (ester C—O).

To a soln of **8** (3·85 g) in DMF (40 ml), NaN_3 (1·56 g) in H_2O (5 ml) was added and the mixture heated for 10 hr at 70°. It was cooled and poured into saturated salt soln (500 ml) which was extracted with two 200 ml portions of ether. Evaporation of the combined ethereal fractions after washing with saturated salt soln gave 2·8 g of the *cis* azide (**10**) as an oily residue. This material proved to be impure (TLC, i-propyl ether) by traces of the starting tosylate and of the trans isomer of the azide. A sample for spectroscopic purposes was purified by preparative TLC. IR ($CHCl_3$), cm^{-1}: 2100 (azide), 1745 (ester C=O), 1700 (urethane C=O), 1595 (aromatic ring), 1165 (ester C—O).

Unpurified **10** (2·4 g) in MeOH (60 ml) was hydrogenated over 340 mg 10% Pd/C at room temp. After 2 hr the catalyst was replaced and hydrogenation repeated. The catalyst was removed, 12 ml 2N NaOH was added and the mixture allowed to react at room temp. After 6 hr 45 ml H_2O was added, MeOH removed *in vacuo* and the soln passed through a column containing 60 ml Dowex 50 W × 4 resin (H^+ form). The column was washed with water and eluted with 1 M NH_4OH. Evaporation of the combined ninhydrin-positive fractions gave an oily residue which proved to be (TLC) *cis*-3-amino-D,L-proline contaminated by the *trans* isomer. To a soln of this material in 20 ml H_2O, 9 ml 1N HCl and 3·3 g of *p*-hydroxy-azobenzen-*p*-sulfonic acid disodium salt in 180 ml of warm water were added. The solid ppt was recrystallized from H_2O to give 1·5 g of **13** *p*-hydroxy-azobenzen-*p*-sulfonate, m.p. 210–212°. A portion of this material was dissolved with water and passed through a column of Amberlite IRA 400 resin (Cl^- form). Evaporation of the ninhydrin-positive fractions gave an oily residue which was crystallized from H_2O–EtOH to give **13** hydrochloride m.p. 161–162° dec. (Found: C, 32·41, H, 6·95; N, 15·23. $C_5H_{11}N_2O_2Cl.H_2O$ requires: C, 32·53; H, 7·10; N, 15·17%).

trans-3-*Amino*-D,L-*proline* (**12**) (azide route). Tosylation of **7** (1 g), was carried out as described for **6** to give 1·56 g (98% yield) of the *cis*-tosylate (**9**) as a thick oil. NMR and IR spectra were taken on a sample purified by column chromatography. IR (liquid film), cm^{-1}; 1745 (ester C=O); 1700 (urethane C=O); 1592 and 1490 (aromatic ring); 1355 and 1205 (tosylate SO_2); 1170 (ester C—O). Reaction of **9** (1·48 g) with NaN_3 was carried out as described for **8** to give 1·03 g of the *trans* azide (**11**) as an oily residue homogeneous by TLC. NMR and IR spectra were taken on a sample purified by column chromatography. IR ($CHCl_3$), cm^{-1}: 2105 (azide), 1745 (ester C=O), 1700 (urethane C=O), 1595 (paromatic ring), 1165 (ester C—O).

770 mg of the *trans*-azide (**11**) were hydrogenated as described for the *cis* isomer (**10**). After removal of the catalyst and evaporation of the solvent, the residue was acidified with HCl and crystallized to give 160 mg of **12** hydrochloride, m.p. 228° dec. (Found: C, 35·88; H, 6·69; N, 16·85. $C_5H_{11}N_2O_2Cl$ requires: C, 36·05; H, 6·66; N, 16·81%).

Methyl 1-*acetyl*-Δ^2-*pirroline*-2-*carboxylate* (**14**). This compound was obtained by methylation of the parent acid[9] with diazomethane in CH_2Cl_2. Evaporation of the solvent and crystallization from i-propyl ether gave **14** (80% yield). m.p. 65–66° (Found: C, 56·70; H, 6·71; N, 8·37; $C_8H_{11}NO_3$ requires: C, 56·80; H, 6·85; N, 8·28%).

trans-N-*Acetyl*-3-*benzylamino*-D,L-*proline methyl ester* (**15**). A soln of **14** (4·5 g) and benzylamine (12·5 ml) in 90% aqueous MeOH (50 ml) was allowed to react for 5 hr at room temp. The solvent was removed *in vacuo* and most of the excess benzylamine in high vacuum on a steam bath. The oily residue dissolved in dry

EtOH and crystallized as the acid oxalate of **15**, yield 5·5 g; m.p. 184–185°. Picrate of **15** (from EtOH); m.p. 164–166° (Found: C, 49·77; H, 4·67; N, 13·92. $C_{21}H_{23}N_5O_{10}$ requires: C, 49·90; H, 4·59; N, 13·86%).

trans-N-*Acetyl*-3-*benzylamino*-D,L-*proline* (**16**). The acid oxalate **15** (3·3 g) was stirred with 2N NaOH (330 ml) for 24 hr at room temp. The soln was washed with CH_2Cl_2, acidified with HCl and taken to dryness *in vacuo*. The solid material was extracted with EtOH and the solvent evaporated. An aqueous soln of the residue was passed through a column containing 70 ml of Dowex 21 K resin (OH^- form). The column was washed with water and eluted with 1 M AcOH. Removal of the solvent from the combined ninhydrin-positive fractions and crystallization from H_2O–acetone gave 1·5 g of **16**, m.p. 146–147° (Found: C, 60·01; H, 7·19; N, 10·01. $C_{14}H_{18}N_2O_3.H_2O$ requires: C, 59·99; H, 7·19; N, 9·99%).

trans-N-*Acetyl*-3-*amino*-D,L-*proline* (**17**). A soln of **16** (1·4 g) in AcOH (40 ml) was hydrogenated with 0·5 g 10% Pd/C at room temp. After 3 hr the catalyst was removed and the solvent evaporated *in vacuo*. The residue was crystallized from H_2O–EtOH to give 720 mg of **17**, m.p. 270° dec. (Found: C, 48·63; H, 7·12; N, 16·06. $C_7H_{12}N_2O_3$ requires: C, 48·83; H, 7·02; N, 16·27%).

trans-3-*Amino*-D,L-proline (**12**). (Addition route). A soln of **17** (250 mg) in 6N HCl (60 ml) was heated on a steam bath for 2 hr. Removal of the solvent and crystallization from H_2O–EtOH gave 190 mg of **12** hydrochloride m.p. 228° dec. (Found: C, 35·91; H, 6·59; N, 17·00; Cl, 21·22. $C_5H_{11}N_2O_2Cl$ requires: C, 36·05; H, 6·66; N, 16·81; Cl, 21·28%).

Oxidation of trans-3-*amino*-D,L-*proline with DAO*. The incubation mixture contained **12** hydrochloride (180 mg), 10 ml 0·1 M potassium pyrophosphate buffer pH 8·3, 25 mg DAO (Boeringher. Suspension in 5 ml aqueous ammonium sulfate) and 1000 units of catalase. The pH was immediately readjusted to 8·3 by careful addition of 2N NaOH and the mixture incubated with stirring at 37° for 24 hr. The protein was removed from the very dark soln with 15% trichloroacetic acid and the excess TCA extracted with ether. An ion-exchange process (Dowex 50 Wx4) preparative TLC (95% EtOH–25% NH_4OH 3 : 1) and crystallization from H_2O–EtOH gave 32 mg of **18** hydrochloride, m.p. 230° dec. (Found: C, 35·70; H, 6·57; N, 16·74. $C_5H_{11}N_2O_2Cl$ requires: C, 36·05; H, 6·66; N, 16·81%).

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