

OCCURRENCE OF cis- AND trans-3-HYDROXY-L-PROLINE IN ACID  
HYDROLYZATE OF TELOMYCIN

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In studies of the amino acid patterns of various species one of us (F. I.) observed that acid hydrolyzates of the antibiotic Telomycin (1) contained two ninhydrin-reactive components that behaved like cyclic imino acids. Isolation of these two amino acids was accomplished by reacting the acid hydrolyzate of Telomycin at pH 7.7 with 2,4,6-trinitrobenzenesulfonic acid (2), separation of the trinitrophenylamino acids from the unchanged cyclic imino acids by passage through Dowex-1 acetate and by subsequent ion-exchange chromatography of the fraction containing the imino acids. The first compound eluted by citrate buffer, pH 3.25, was the trans-3-hydroxy-L-proline identical with that isolated from sponge (3). One gram of Telomycin yielded 31 mg. of the trans isomer in crystalline form from ethanol-H<sub>2</sub>O.

Anal. Calcd. for C<sub>5</sub>H<sub>9</sub>NO<sub>3</sub>: C, 45.80; H, 6.92; N, 10.68.

Found: C, 45.88; H, 6.99; N, 10.60.

By ion-exchange chromatography, by paper chromatography in five solvent systems, by high voltage paper electrophoresis and by color reactions with ninhydrin, isatin and β-naphthoquinone-4-sulfonate the isolated trans isomer behaved exactly as that from sponge which was identified as trans-3-hydroxy-L-proline (3). The L-configuration was likewise assigned to this isomer based on its non-reactivity towards D-amino acid oxidase.

The cis isomer was also eluted from the column with pH 3.25 buffer and emerged much later than the trans isomer. It was obtained in crystalline form (33 mg. from 1 g. of Telomycin) from ethanol-H<sub>2</sub>O.

Specific rotation  $[\alpha]_D^{20}$   $-91.5^\circ \pm 1.5^\circ$  ( $c = 0.6\%$  in  $H_2O$ ).

Anal. Calcd. for  $C_5H_9NO_3$ : C, 45.80; H, 6.92; N, 10.68.

Found: C, 45.68; H, 6.68; N, 10.64.

On the automatic amino acid analyzer (Phoenix) the peak due to the cis isomer overlapped that of threonine. Like the trans isomer the cis compound gave a yellow color with ninhydrin which absorbs maximally at 440  $m\mu$ .

The methyl ester of N-carbobenzyloxy-trans-3-hydroxy-DL-proline (3) in acetone was oxidized with 8N  $CrO_3$  (4) giving the 3-oxo compound which failed to crystallize. However, gas chromatography as well as thin layer chromatography showed the compound to be homogeneous. Moreover, an IR spectrum of the compound revealed the characteristic band at 5.63  $\mu$  attributable to the five-membered ketone.

Reduction of the ketone with sodium borohydride in dimethoxyethane followed by hydrolysis and subsequent catalytic hydrogenation over palladium (5) yielded a mixture of cis-(37%) and trans-(63%) 3-hydroxyprolines. Ion-exchange chromatography was employed to separate the diastereoisomers (3). The cis-3-hydroxy-DL-proline was obtained in crystalline form from ethanol- $H_2O$ .

Anal. Calcd. for  $C_5H_9NO_3$ : C, 45.80; H, 6.92; N, 10.68.

Found: C, 45.83; H, 6.94; N, 10.66.

The synthetic and natural cis-3-hydroxyprolines proved to be inseparable by paper chromatography in five solvent systems, by ion-exchange chromatography and by high voltage paper electrophoresis. The D-isomer of the racemate was oxidized quantitatively by D-amino acid oxidase.

Previous work on the amino acid composition of Telomycin showed that it contained aspartic acid, threonine, glycine and alanine (1). Recently, erythro- $\beta$ -hydroxy-L-leucine was proved to be a component of Telomycin (6). With the use of the automatic amino acid analyzer we found that Telomycin has the

following amino acid composition:

	<u>Molar ratio</u>
Alanine	0.97
Aspartic acid	0.99
Glycine	1.00
$\beta$ -Hydroxy-leucine	0.63
<u>cis</u> -3-Hydroxyproline	0.69
<u>trans</u> -3-Hydroxyproline	0.69
Serine	0.87
allo-Threonine	0.93
Threonine	0.93
Amide Ammonia	0.61

Treatment of the separate isomeric 3-hydroxyprolines with constant boiling HCl for 20 hrs. at 110° results in approximately 30 % destruction of each isomer. In neither case is there an interconversion of the forms. We believe, therefore, that the cis and trans isomers occur as such in Telomycin.

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