ISOLATION AND SYNTHESIS OF 3-HYDROXY-L-PROLINE

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Chromatograms of the hydrolysates of vertebrate and invertebrate collagens showed an unknown peak in front of hydroxyproline and adjacent to methionine sulfoxides (1). Degradation of collagen by the exoenzymes of Clostridium histolyticum resulted in the liberation of several peptides, one of which contained glycine, an unknown amino acid and hydroxyproline (2). Paper and column chromatographic studies by one of us (F. I.) of the hydrolysates of collagen and of sponge from various sources revealed an unknown component in the vicinity of 4-hydroxyproline, and yielded color reactions similar to those from the cyclic imino acids (3).

This report deals with the isolation of this unknown component from sponge, its synthesis and identification as 3-hydroxy-L-proline.

The acid hydrolysate of Mediterranean sponge was nitrosated (4) and the mixture of nitroso derivates of cyclic imino acids isolated and hydrolyzed. The cyclic imino acids were separated by ion-exchange chromatography on IR-120 resin (5). The combined material from eleven column runs representing 64 g. of dried sponge was crystallized from aqueous ethanol, yielding 62 mg. of the amino acid. M. P., decomposes above 200°. Specific rotation $[\alpha]_D^{20}$ -24.4° (c = 0.5%).

Anal. Calcd. for $C_5H_9NO_3$: C, 45.80; H, 6.92; N, 10.68.
Found: C, 45.82; H, 6.74; N, 10.46

On the automatic amino acid analyzer (Phoenix) the peak due to this unknown occurs at 95 ml. when the run was made at 30→50° and the ninhydrin color has a maximum absorption at 440 mμ similar to those of hy-

droxyproline and proline. On a 2-dimensional paper chromatogram (6) it occupies a position just above that of 4-hydroxyproline.

The methyl ester of N-carbobenzyloxy-3,4-dehydro-DL-proline (7) was reacted with diborane (8), the resulting alkylborane oxidized with alkaline hydrogen peroxide and product hydrogenolyzed to a mixture of isomeric 3- and 4-hydroxyprolines. On the automatic amino acid analyzer the mixture was resolved into 68% 3-hydroxyproline, 10% 4-hydroxyproline and a trace of 4-allo-hydroxyproline. The 3-hydroxyproline fraction was purified by column chromatography (5) as well as by fractional crystallization from aqueous ethanol. M. P. decomposes above 200°.

Anal. Calcd. for $C_5H_9NO_3$: C, 45.80; H, 6.92; N, 10.68.
Found: C, 46.01; H, 6.98; N, 10.62.

The amino acid proved to be homogenous in a number of systems capable of resolving the isomeric 4-hydroxyprolines, indicating that only one geometrical isomer is formed in the hydroboration reaction. Since 4-hydroxyproline is formed almost to the exclusion of the allo isomer, we feel that stereochemical considerations of the hydroboration reaction (8) point to the fact that the 3-hydroxyproline also belongs to the normal or trans series.

The synthetic amino acid and the natural compound (from sponge) proved to be inseparable by paper chromatography in five solvent systems, by ion-exchange chromatography and by high voltage paper electrophoresis. Infrared spectra of the N-carbobenzyloxyamino acid methyl esters were identical and the two O-acetyl derivatives could not be separated by gas chromatography.

The D-isomer was oxidized quantitatively in the racemate by D-amino acid oxidase. Since the natural amino acid was unchanged under the same condition, it would appear to be of the L-configuration.

We have also chromatographically identified the same amino acid in collagen hydrolysates.

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