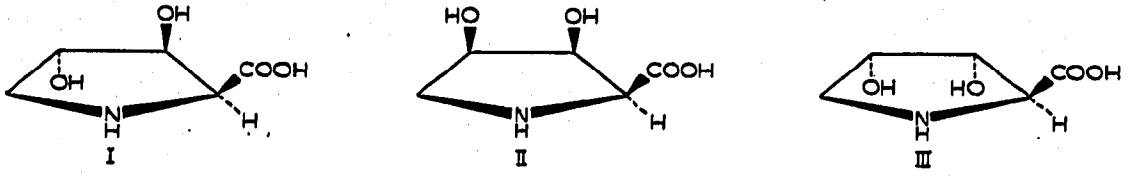
Notes

CHROM. 4149

Identification and characterization of natural and synthetic dihydroxyprolines

We have previously reported on the position of the cyclic secondary amino acids on the automatic amino acid analyzer[1]. At that time only monohydroxy derivatives of pyrrolidine and piperidine carboxylic acids were known. Recently the first natural occurrence of a 3,4-dihydroxy-L-proline was reported[2]. Complete X-ray analysis[3] and mass spectrometry[3] confirmed and established the configuration of this new amino acid as 2,3-*cis*-3,4-*trans*-3,4-dihydroxy-L-proline (I). Although the existence of a dihydroxy-L-proline in nature had been anticipated[4], synthetic attempts have so far yielded only 2,3-*cis*-3,4-*cis*-3,4-dihydroxy-DL-proline (II, only L-form shown) and 2,3-*trans*-3,4-*cis*-3,4-dihydroxy-DL-proline (III, L-form shown) by glycolization[5] of 3,4-dehydro-DL-proline[6].



We have now established the sequence of elution of I, II and III in a composite

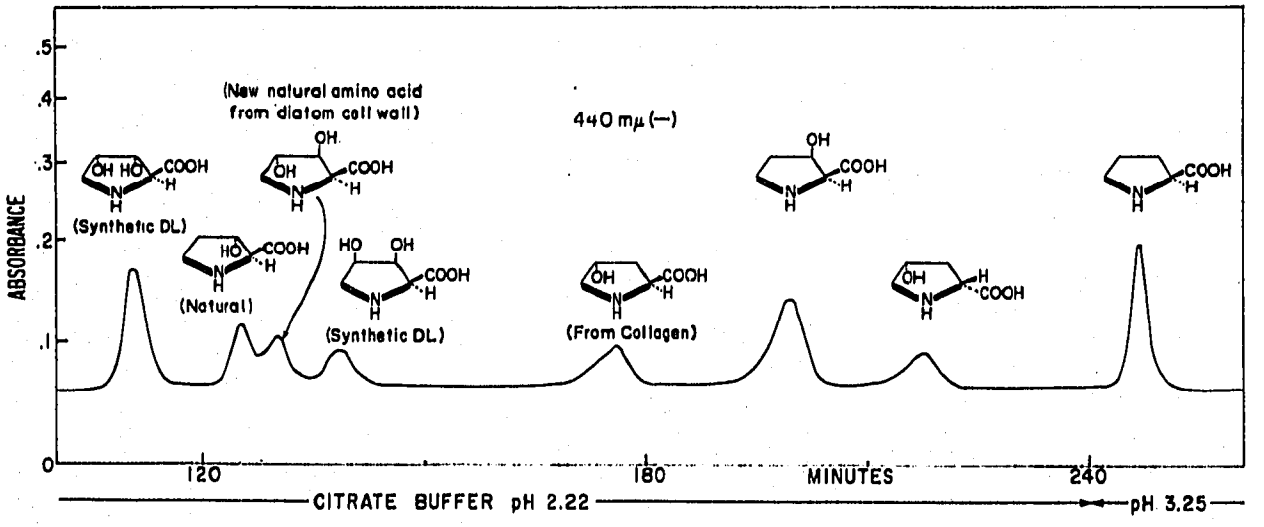


Fig. 1. Sequence of elution of 3,4-dihydroxy- and 3- and 4-monohydroxyprolines on the automated amino acid analyzer. Column size 69 × 0.9 cm; resin, Beckman Type UR-30; height of resin column 56 cm; column flow rate 68 ml/h; first buffer, citrate (0.2 *N*) pH 2.20; second buffer, citrate (0.2 *N*) pH 3.25; buffer change time 2 h 35 min; temperature 55.5°; ninhydrin color values were read at λ = 440 mμ. For key see Table I.

TABLE I

COMPOUNDS IN FIG. 1 AND THEIR NINHYDRIN VALUES

Compounds	*Amount applied (μm)*
(1) 2,3-*trans*-3,4-*cis*-3,4-Dihydroxy-DL-proline	0.14
(2) *trans*-3-Hydroxy-L-proline	0.10
(3) Natural 2,3-*cis*-3,4-*trans*-3,4-dihydroxyproline	0.05
(4) 2,3-*cis*-3,4-*trans*-3,4-Dihydroxy-DL-proline	0.03
(5) *trans*-4-Hydroxy-L-proline	0.10
(6) *cis*-3-Hydroxy-L-proline	0.20
(7) *allo*-4-Hydroxy-D-proline	0.10
(8) L-Proline	0.10

TABLE II

HIGH VOLTAGE PAPER ELECTROPHORESIS OF THE 3,4-DIHYDROXYPROLINES I, II AND III

Buffer: formic acid pH 1.81; voltage: 4000 V; amperage: 135 mA; length of run: 2 h.

Compound	*Distance traveled towards negative pole (mm)*
2,3-*cis*-3,4-*trans*-3,4-Dihydroxy-L-proline (I)	236
2,3-*cis*-3,4-*cis*-3,4-Dihydroxy-DL-proline (II)	231
2,3-*trans*-3,4-*cis*-3,4-Dihydroxy-DL-proline (III)	196

mixture containing *cis*- and *trans*-3- and 4-hydroxyprolines from a 56-cm column of an automated amino acid analyzer (Phoenix) under conditions described in the legend to Fig. 1. The amounts applied are given in Table I.

Table II lists the mobility of the dihydroxyprolines I, II and III on high voltage paper electrophoresis.

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