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THE STEREOCHEMISTRY OF 4-HYDROXYMETHYLPROLINE

K. G. Untch and G. A. Gibbon
Mellon Institute, Pittsburgh, Pennsylvania 15213

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An amino acid, isolated independently from apple peels by Hulme (1) and from apple twigs by Urbach (2), has been proved to be 4-hydroxymethylproline by two independent investigations utilizing n.m.r. (3) and mass spectroscopy (4). However, the stereochemistry of this amino acid remains in doubt.

Four groups of investigators have obtained data that indicate its configuration. Its proton n.m.r. spectrum (3) resembles cis-more than that of trans-4-methylproline (known absolute configuration (5)). A stereoselective synthesis of 4-hydroxymethyl-L-proline by Kenner and co-workers (6) provides results, though not conclusive, favor the cis-L-configuration. On the other side, Biemann, et. al. (4) state that the amino acid is established as trans-4-hydroxymethylproline based on the fact that the mass spectrum of its ethyl ester remains unchanged after 30 min. at 140° at high vacuum and the argument that the cis isomer would have lactonized under these conditions. A synthesis of (±)-4-hydroxymethylproline by Burgstahler and Aiman (7) gave the DL-compound whose ethyl ester gave an identical mass spectrum to that of the natural amino acid and they favor the trans-configuration. Finally, the trans assignment may be thought to be more reasonable since 4-hydroxymethylproline is accompanied by trans-4-methylproline in apples (5).

In order to obtain 4-hydroxymethyl-L-proline for a biochemical study, we repeated its stereoselective synthesis from 4-hydroxy-L-proline following the scheme of Bethell, Bigley and Kenner (6). The benzhydryl ester of carbobenzyloxy-4-methylene-L-proline (I) when reacted with disiamylborane (8), followed by oxidation with alkaline aqueous hydrogen peroxide gave a mixture of two benzhydryl esters of 4-hydroxymethyl-L-proline (ca. 80% crude yield). Separation of these benzhydryl esters was achieved by a combination of fractional crystallization and column chromatography ($SiO_2 \cdot xH_2O$) and gave a solid (II)* (ca. 70%), m.p. 159.5-160.5°(corrd.), $[\alpha]_D^{23}$ -61° ($CHCl_3$) (lit. 159-160°, -58°), and a solid (III) (ca. 30%), m.p. 105-113°**, $[\alpha]_D^{23}$ -44° ($CHCl_3$). Hydrogenolysis of II in tetrahydrofuran with 10% palladium on charcoal gave 4-hydroxymethyl-L-proline (IV), m.p. 255-257° (decomp.) (lit. 257-8°) whose infrared and proton n.m.r. spectra were identical to those of the natural amino acid (1,3). Hydrogenolysis of III in ethanol with 10% pallidium on charcoal afforded the isomeric 4-hydroxymethyl-L-proline (V), m.p. 227.5-229°, $[\alpha]_{589.6}$ -48° (H_2O, obtained from an O.R.D. curve). The proton n.m.r. spectra of these two 4-hydroxymethyl-L-prolines resembled those of cis and trans-4-methylproline (5), pairwise. As previously pointed out by Abraham et. al. (3), the proton n.m.r. spectrum of the natural 4-hydroxymethylproline resembles cis- rather than trans-4-methylproline. Our synthetic isomer, m.p. 227.5-229°, exhibits an n.m.r. spectrum similar to trans- rather than cis-4-methylproline. Compare the

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* Satisfactory analyses were obtained for all compounds reported.

** The melting behavior of this compound is variable and samples that had been repeatedly recrystallized exhibited at least a 7° melting range.

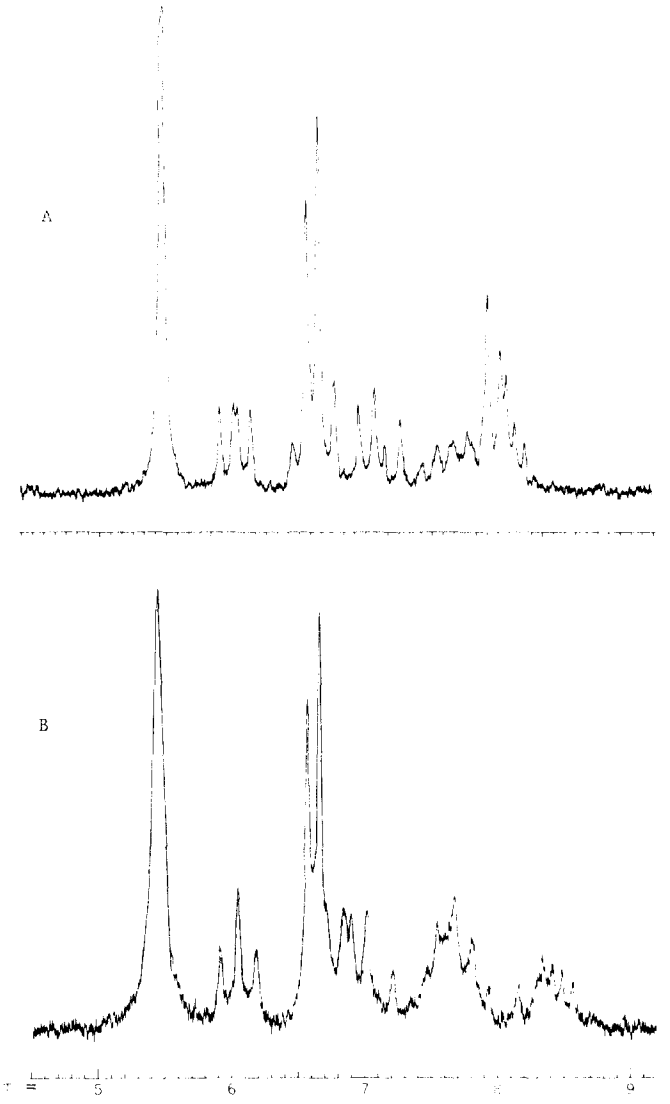
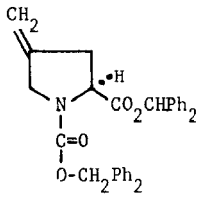


Fig. 1. — Proton n.m.r. spectra of (A) trans-4-hydroxymethyl-L-proline and (B) cis-4-hydroxymethyl-L-proline in deuterium oxide solution (Varian Associates A-60 spectrometer).

n.m.r. spectra, Fig. 1, to those of the 4-methylprolines (3). Thus, this correlation of the n.m.r. spectra of both isomeric 4-hydroxymethyl-L-prolines to those of cis and trans-4-methylprolines strongly suggests that the natural amino acid is cis-4-hydroxymethyl-L-proline. Consistent with the natural amino acid having structure IV is the method of synthesis. The expectation that the dialkylborane would attack the olefin preferentially from the less hindered side, affording the adduct that would result in the cis-amino acid (6), seems to have been realized since the isomer that predominates (ca. 70%) is identical to the natural amino acid and the other less abundant isomer corresponds to a 4-hydroxymethyl-L-proline having the trans configuration.



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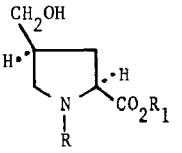
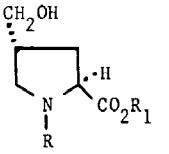


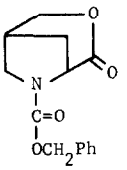
II, R = CO_2CH_2Ph
R_1 = $CHPh_2$
IV, R and R_1 = H
VI, R = CO_2CH_2Ph
R_1 = H



III, R = CO_2CH_2Ph
R_1 = $CHPh_2$
V, R and R_1 = H
VII, R = CO_2CH_2Ph
R_1 = H

In order to prove rigorously the stereochemistry of 4-hydroxymethyl-L-proline both IV and V were converted to their N-carbobenzyloxy derivatives, respectively, giving VI, a white solid, m.p. 115.5-116.5°, $[\alpha]_D^{23}$ - 48.5°, whose infrared spectrum showed absorption at 1705 $cm.^{-1}$, and an oil, (VII), which resisted all attempts to obtain a crystalline

sample (purified by column chromatography on $SiO_2 \cdot xH_2O$), whose infrared spectrum closely resembled that of VI. Each isomer, VI and VII, was treated with dicyclohexylcarbodiimide in acetonitrile. VI afforded the crystalline lactone, VIII (ca. 33%), m.p. 94.5-95.5°, whose infrared spectrum showed absorption at 1755 and 1705 $cm.^{-1}$. VII, when treated identically with dicyclohexylcarbodiimide, resulted in recovery of the starting material (60%) and no trace of a lactone (some of VII was recovered as its acylurea derivative, ca. 30%).



VIII

These data allow a conclusive stereochemical assigment to be made for the natural amino acid. It is cis-4-hydroxymethyl-L-proline, structure **IV**. All earlier data (3,4,6) obtained on this amino acid are consistent with this result and only the lack of any lactonization of its ethyl ester, under the conditions employed by Biemann and co-workers (4), remains as somewhat surprising. It is interesting that cis-4-hydroxymethyl-L-proline accompanies trans-4-methylproline in the natural source (5).

*** After the submission of this paper, A. Burgstahler has informed us by a private communication that he has prepared a non-crystalline lactone corresponding to ours from his DL-4-hydroxymethylproline (7).

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