Tetrahedron Letters,Vol.26,No.3,pp 351-354,1985
Printed in Great Britain

0040-4039/85 $3.00 + .00

ELUCIDATION OF STEREOSPECIFICITY OF A SELENIUM-CONTAINING HYDROGENASE FROM *METHANOCOCCUS VANNIELII* — SYNTHESES OF *(R)*- AND *(S)*-[4-$^2H_1$]-3,4-DIHYDRO-7-HYDROXY-1-HYDROXYETHYLQUINOLINONE

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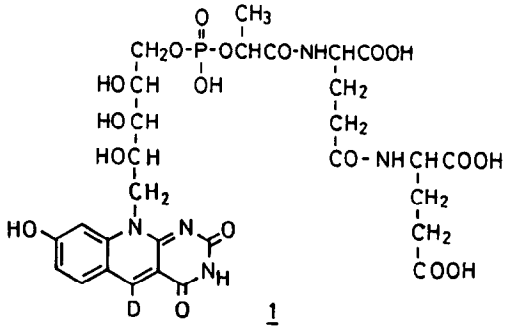
Summary: To elucidate the stereospecificity of the selenium-containing hydrogenase from *Methanococcus vannielii*, *(R)*- and *(S)*-[4-$^2H_1$]-3,4-dihydro-7-hydroxy-1-hydroxyethylquinolinone were synthesized as authentic samples for comparison with the compound which was derived from the enzymic reaction product.

The 8-hydroxy-5-deazaflavin cofactor (F_{420}) was first isolated from *Methanobacterium* strain MOH[1] and its structure 1 was established as a 5-deazaflavin derivative.[2] The only known 5-deazaflavin cofactor specific enyzme, 8-hydroxy-5-deazaflavin-dependent $NADP^+$ reductase has been isolated from *Methanococcus vannielii*[3] permitting the studies of the structure-reactivity relationships of several 5-deazaflavin analogs for this enzyme[4], as well as the stereochemistry of the enzyme reaction[5]. A selenium-containing hydrogenase which catalyzes the reduction of 5-deazaflavin derivatives with molecular hydrogen has also been isolated from *M. vannielii* by Yamazaki[6]. It is found that the $NADP^+$ reductase oxidizes the reduced 5-deazaflavin with abstraction of the same hydrogen atom that is added at C-5 of 5-deazaflavin upon reduction by the hydrogenase.[7] Further evidence that the enzymic reduction by the hydrogenase occurs stereospecifically was furnished by



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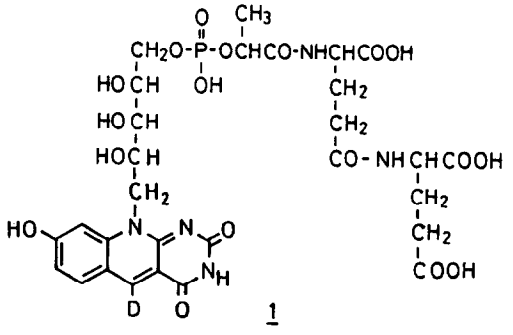

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demonstrating that the reduction product of [5-$^2H_1$]-7,8-didemthyl-8-hydroxy-5-deazariboflavin by the hydrogenase gave an optically active [4-$^2H_1$]-3,4-dihydro-7-hydroxy-1-hydroxyethylquinolinone after the successive modification of the molecule as shown in Figure 1[8]. In order to determine the absolute configuration at the C-5 prochiral center of the deuterium-labeled product and to establish the stereochemical course of the enzymic reaction, it is necessary to prepare authentic *(R)*- and *(S)*-[4-$^2H_1$]-3,4-dihydro-7-hydroxy-1-hydroxyethylquinolinones. This paper reports the successful synthesis of these compounds from *(S)*- and *(R)*-3-hydroxy-3-phenylpropionic acid[9].

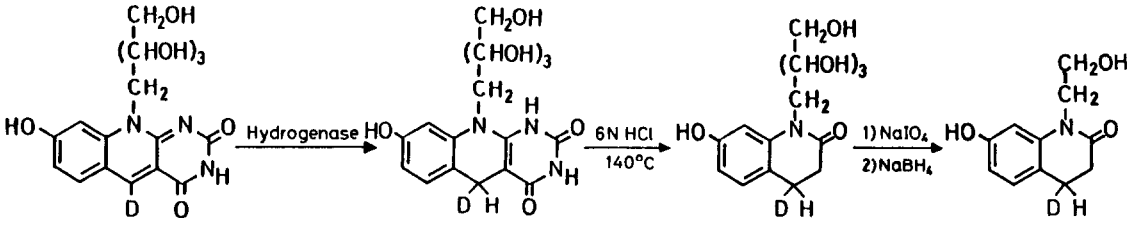

Fig. 1

As a chiral deutero intermediate in this synthesis, *(R)*-[3-$^2H_1$]-3-phenylpropionic acid was synthesized as shown in Figure 2. For conversion of the hydroxyl group of 2 to deuterium, lithium aluminum deuteride reduction of halide was used. First *(S)*-3-hydroxy-3-phenylpropionic acid (2) which was obtained by optical resolution with brucine[10] was reduced to the alcohol (3) by esterification followed by sodium borohydride reduction. This step was essential because otherwise the dehydration reaction occurred readily. The primary hydroxyl group was selectively blocked with a pivaloyl group to give the compound (4). The secondary hydroxyl group of 4 was converted to the chloride by reaction with thionylchloride with retention of the configuration to afford 5, which was then reduced by lithium aluminum deuteride with inversion of the configuration at the asymmetric carbon atom. The deutero derivative (6) thus obtained was oxidized to give *(R)*-[3-$^2H_1$]-3-phenylpropionic acid (7) (mp 47.5-49°C, $[\alpha]_D^{18}$ - 0.33° (c 9.98, MeOH)). The enantiomer, *(S)*-[3-$^2H_1$]-3-phenylpropionic acid (7') was also synthesized in the same manner starting from *(R)*-[3-$^2H_1$]-3-hydroxy-3-phenylpropionic acid.

In order to confirm the assigned configuration, the compound (7) and its

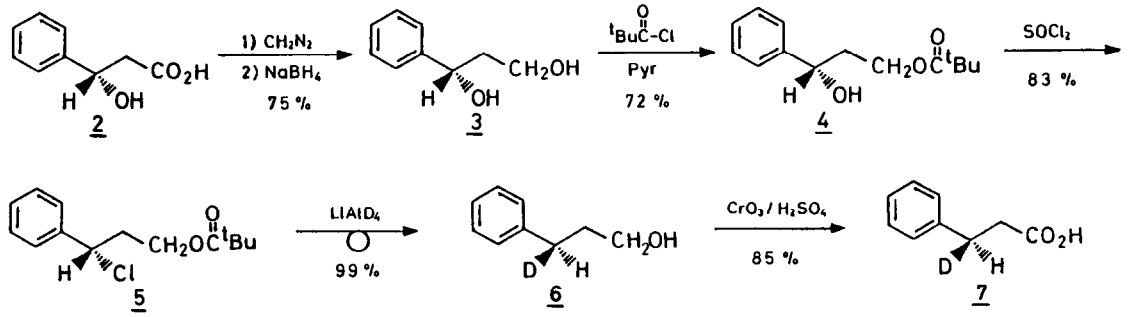

Fig. 2

enantiomer (7') were converted to [2-$^2H_1$]-succinic acid whose absolute configuration was already established[11]. Thus, the compound (7), and (7') were oxidized by ruthenium trichloride and sodium hypochlorite[12] to give *(R)*- and *(S)*-[2-$^2H_1$]-succinic acid (8), and (8') respectively as shown in Figure 3, where the compound (8) showed minus while (8') showed plus optical rotation. As *(R)*-[2-$^2H_1$]-succinic acid was known to show minus optical rotation[11], the configuration of 8 was proven to be *(R)*. From this fact, the compound (7) obtained by the above procedure was verified to be *(R)*-[3-$^2H_1$]-3-phenylpropionic acid.

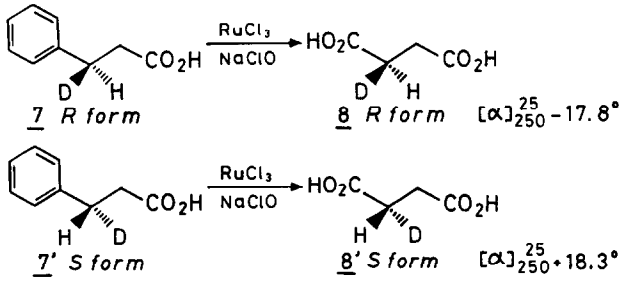

Fig. 3

In a series of the reactions to connect the authentic [3-$^2H_1$]-3-phenylpropionic acid with the enzymic product, [4-$^2H_1$]-3,4-dihydro-7-hydroxy-1-hydroxyethylquinolinone, *(R)*-[3-$^2H_1$]-3-phenylpropionic acid (7) was first converted to the dinitro derivative (9) which was reduced by zinc powder in hydrochloric acid to give the lactam derivative (10) (mp 181-184°C, $[\alpha]^{24}_{350}$ +5.3° (*c* 5.24, 1 N HCl)) as shown in Figure 4. For conversion of the amino group to a hydroxyl group on the benzene ring, 10 was treated with nitrous acid to give 11 (mp 212-215°C, $[\alpha]^{21}_{400}$ +3.3° (*c* 1.22, EtOH). After protection of the phenolic hydroxyl group of 11 with a benzyl group, N-alkylation of 12 with ethyl bromoacetate afforded the derivative (13) (mp 68-69°C, $[\alpha]^{23}_{350}$ +6.5° (*c* 3.07, MeOH)). Reduction of 13 by sodium borohydride followed by hydrogenation gave *(R)* enantiomer which showed plus optical rotation while the *(S)* enantiomer obtained from *(S)*-[3-$^2H_1$]-3-phenylpropionic acid by the same procedure showed minus optical rotation as shown in Figure 5.

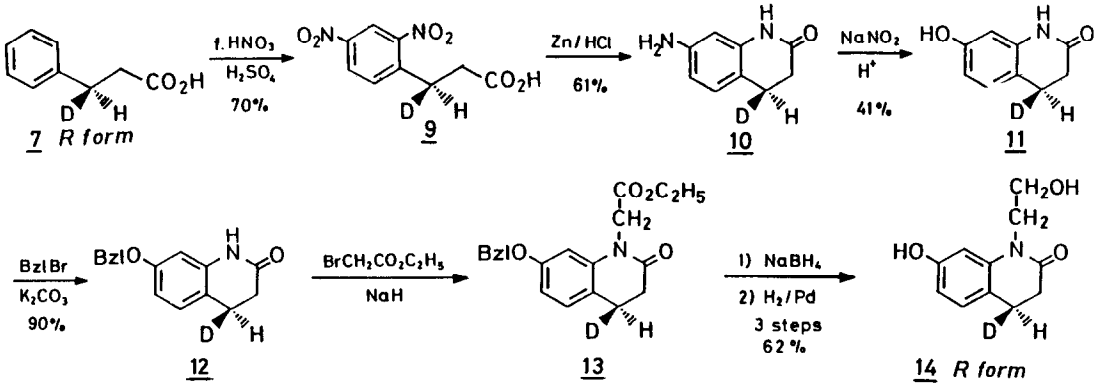

Fig. 4