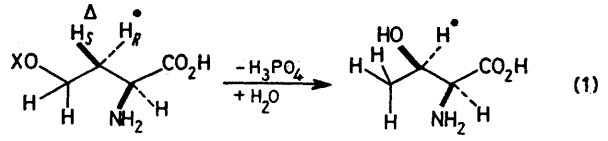
Stereochemistry of the Hydrogen Removal at Position 3 in the Enzymic Synthesis of L-Threonine from *O*-Phospho-L-homoserine

By CLAUDIO FUGANTI
(*Istituto di Chimica del Politecnico*, 20133 *Milano, Italy*)

Summary Experiments with (3*R*)- and (3*S*)-[3-^{3}H; *U*-^{14}C]-*O*-phospho-L-homoserine, (**1a**) and (**1b**), show that the conversion into L-threonine (**2**) effected by yeast enzyme proceeds with loss of the 3-*pro-S* and retention of the 3-*pro-R* hydrogen atoms.

THE biological synthesis[1] of the amino acid L-threonine (**2**) proceeds from *O*-phospho-L-homoserine (**1**) according to equation (1). This transformation, catalysed by a pyridoxal phosphate (PLP)[2] dependent enzyme, formally involves non-hydrolytic removal of the phosphate group, accompanied by the addition of a hydroxy group to give L-threonine (**2**). We here describe the stereochemical analysis of the transformation indicated in equation (1), using homoserine samples asymmetrically labelled with isotopic hydrogen in the 3-position.[3,4]



(1)

(1a) $X = H_2PO_3$; $H_R = {}^3H$; $H_S = {}^1H$

(1b) $X = H_2PO_3$; $H_R = {}^1H$; $H_S = {}^3H$

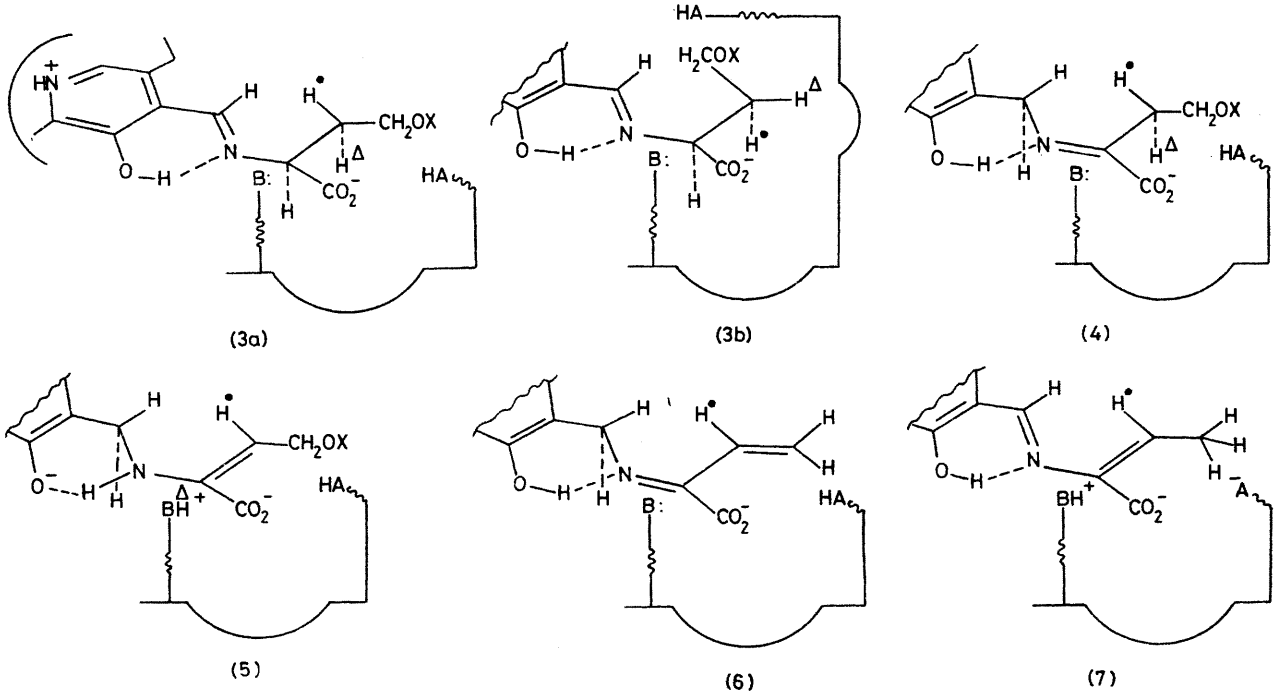
(2)

(3*R*)-[3-^{3}H]-L-Homoserine was prepared from [α-^{3}H] cinnamic acid, as reported for the deuteriated series.[3] This material, mixed with [*U*-^{14}C]-L-homoserine, was converted[5] into (3*R*)-[3-^{3}H; *U*-^{14}C]-*O*-phospho-L-homoserine (**1a**), without tritium loss. The procedure[3] used in the synthesis of (3*S*)-[3-^{2}H]-L-homoserine could not be applied in the scaled-down tritium work, therefore (3*S*)-[3-^{3}H]-D,L-homoserine was prepared as follows. Reduction of [α-^{3}H]cinnamaldehyde with fermenting baker's yeast yielded [2-^{3}H]-3-phenylpropanol, which, on the basis of studies in the deuteriated series,[6] should contain *ca.* 90% of the 2*S*-isomer. This asymmetrically labelled material was converted, according to the described procedures,[3] into (3*S*)-[3-^{3}H]-D,L-homoserine, mixed with [*U*-^{14}C]-L-homoserine, and transformed into [3-^{3}H; *U*-^{14}C]-*O*-phospho-L-homoserine, containing *ca.* 90% of the (3*S*)-isomer (**1b**), using yeast homoserine kinase, which is specific for the L-isomer.[7]

Conversion of the two asymmetrically labelled samples (**1a**) and (**1b**) into L-threonine (**2**) was achieved using a 55% $(NH_4)_2SO_4$ saturation protein fraction from baker's yeast in 0·5 M glycylglycine–KOH buffer, pH 7·3, at 35 °C. The L-threonine (**2**) formed in the enzymic reaction from the (3*R*)-isomer (**1a**), and isolated after dilution with inactive material, showed *ca.* 95% tritium retention. The following experiments show that the ^{3}H in the biosynthetic sample was present at position 3 of (**2**). Metaperiodate oxidation of labelled (**2**) gave acetaldehyde (bearing hydrogen atoms at positions 3 and 4) which was isolated as the dimedone derivative, with the same molar ^{3}H specific activity as the starting material, whereas Kuhn–Roth oxidation gave acetic acid (hydrogen atoms at position 4) devoid of tritium activity.

Enzymic conversion of the (3*S*)-isomer (**1b**) into (**2**) occurred with *ca.* 85% tritium loss, a value which corresponds to almost complete loss of the 3-*pro-S* hydrogen atom of (**1**), if we take into account the optical purity of the precursor. Lack of material did not allow degradation, therefore further work is needed, possibly with material of better optical purity, in order to clarify the possible occurrence[8] of an intramolecular hydrogen shift to the 4-position of (**2**).

The results may have the following mechanistic implication. The key intermediate in the PLP-dependent enzymic reaction is thought[9] to be the Schiff base formed by enzyme-bound PLP with (**1**), which is expected to have either conformation (**3a**) or (**3b**). The two conformations should have the following common features: (i) the C_α–H, C_β–H, and C_γ–OPO_3H_2 σ bonds must be perpendicular to the extended π system in order to optimize orbital interactions in the bond breaking and making processes, and (ii) H_α and one of the stereoheterotopic β hydrogen atoms must be located on the same side of the plane of the enzyme-bound PLP ring in order to allow a single base to be used in the proton removal.[10] The present results, showing the loss of the β *pro-S* hydrogen atom (H^Δ), point to a pathway starting from (**3a**) rather than (**3b**), and continuing *via* the imine (**4**) and the enamine (**5**), which eliminates the γ-substituent together with a proton, to give the vinylglycine derivative (**6**). The latter picks up a proton from the γ-methylene group yielding the *E*-aminocrotonate (**7**),



which, upon water addition, possibly assisted by the same basic group A^-, gives rise, eventually, to L-threonine (**2**) with overall retention of configuration. The last feature is in agreement with other results[11] in the same set of enzymic reactions and with the general view[10] on the significance of enzyme stereospecificity.

(*Received*, 21*st November* 1978; *Com.* 1257.)

[1] Y. Watanabe and K. Shimura, *J. Agric. Chem. Soc. Japan*, 1954, **28**, 678; M. Flavin and C. Slaughter, *Biochem. Biophys. Acta*, 1959, **36**, 554.
[2] E. E. Snell and S. J. Di Mari, in 'The Enzymes,' 3rd Edn., Vol II, ed. P. D. Boyer, Academic Press, New York-London, 1970, p. 335; C. Yanofsky and I. P. Crawford, *ibid.*, Vol. VII, 1972, p. 1.
[3] D. Coggiola, C. Fuganti, D. Ghiringhelli, and P. Grasselli, *J.C.S. Chem. Comm.*, 1976, 143.
[4] C. Fuganti, D. Ghiringhelli, and P. Grasselli, *Experientia*, 1978, **34**, 297.
[5] M. Flavin and C. Slaughter, *J. Biol. Chem.*, 1960, **235**, 1103.
[6] C. Fuganti, D. Ghiringhelli, and P. Grasselli, *J.C.S. Chem. Comm.*, 1975, 846.
[7] Y. Watanabe, S. Konishi, and K. Shimura, *J. Biochem.*, 1957, **44**, 299.
[8] B. I. Posner and M. Flavin, *J. Biol. Chem.*, 1972, **247**, 6412, 6418.
[9] M. Flavin and C. Slaughter, *J. Biol. Chem.*, 1960, **235**, 1112.
[10] R. K. Hanson and I. A. Rose, *Accounts Chem. Res.*, 1975, **8**, 1; and in 'Applications of Biochemical Systems in Organic Chemistry,' eds. B. J. Jones, C. J. Sih, and D. Perlman, Vol. II, Wiley, New York-London, 1976, p. 507.
[11] C. Fuganti and D. Coggiola, *Experientia*, 1977, **33**, 847.