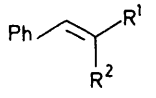
Synthesis of Homoserine Samples Stereospecifically Labelled with Isotopic Hydrogen in the β- and γ-Positions

By DIANELLA COGGIOLA, CLAUDIO FUGANTI,* DARIO GHIRINGHELLI, and PIERO GRASSELLI
(*Istituto di Chimica del Politecnico, Centro del CNR per la Chimica delle Sostanze Organiche Naturali* 20133 *Milano, Italy*)

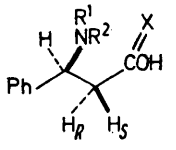
Summary (βR)[β-^{2}H]-L-Homoserine (**17**) and the (βS)-isomer (**8**) are synthesised through a sequence involving as key step the formal *cis* addition of hydroxylamine onto cinnamic acid to form 3-amino-3-phenylpropionic acid; (γR)[γ-^{2}H]-DL-homoserine (**20**) and the (γS)-isomer (**19**) are obtained from the enantiomeric forms of stereospecifically labelled 3-phenyl[1-^{2}H]propanol.

THE amino-acid L-homoserine is a key intermediate in the conversion of L-aspartic acid into L-threonine, L-homocysteine, and α-ketobutyrate in microbia and fungi.[1] This set of transformations catalysed by different pyridoxal phosphate-dependent enzymes is thought[2] to proceed through the intermediacy of an enzyme-bonded vinylglycine derivative arising by elimination of one of the stereoheterotopic[3] protons from the prochiral centre in the β-position together with the γ-substituent from the Schiff's base formed between a suitable *O*-derivative of homoserine and pyridoxal phosphate. Addition of the sulphur nucleophile onto the γ methylene group gives rise, by formal reversal of the reaction pathway, to homocysteine, whereas proton addition gives rise to enzyme-bonded α-aminocrotonate, the precursor of L-threonine and α-ketobutyrate. In view of the present interest[4] in the stereospecificity of enzyme reactions we undertook a stereochemical analysis of the above mentioned set of enzymic transformations.

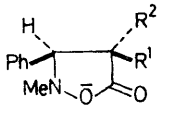
We report now on the synthesis of homoserine samples asymmetrically labelled with isotopic hydrogen in the β- and γ-positions.



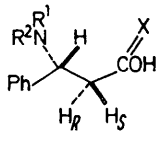
(1) R^1 = CO_2H; R^2 = D
(2) R^1 = CO_2H; R^2 = H
(3) R^1 = H; R^2 = CO_2Et
(4) R^1 = CO_2Et; R^2 = H
(5) R^1 = CO_2NHMe, R^2 = H
(6) R^1 = CO_2Et; R^2 = D



(9) R^1 = R^2 = H_S = H; H_R = D; X = O
(10) R^1 = R^2 = H_R = H; H_S = D; X = O
(11) R^1 = R^2 = Me; H_R = H; H_S = D; X = O
(12) R^1 = R^2 = H_S = H; H_R = D; X = H_2
(13) R^1 = R^2 = H_R = H; H_S = D; X = H_2

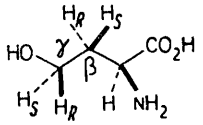


(7) R^1 = R^2 = H
(8) R^1 = H; R^2 = D

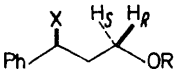


(14) R^1 = R^2 = H_R = H; H_S = D; X = O
(15) R^1 = R^2 = H_S = H; H_R = D; X = O
(16) R^1 = R^2 = Me; H_S = H; H_R = D; X = O

Addition[5] of hydroxylamine in ethanol to (*E*)-[α-^{2}H]-cinnamic acid (**1**) (*ca.* 95% $^2H_1$) affords 3-amino-3-phenyl-[2-^{2}H]propionic acid [(**9**) and (**14**)], showing ^{1}H n.m.r. signals (CF_3CO_2H; 100 MHz) due to the side chain protons at δ 4·98 and 3·5 in an AX pattern, J_{AX} 9·5 Hz, whereas under conditions in which all the exchangeable hydrogen in the reactants and in the solvents had been substituted for deuterium the diastereoisomers [(**10**) and (**15**)] are obtained from (*E*)-cinnamic acid (**2**), showing a BX pattern at δ 4·98 and 3·25 with J_{BX} 4·0 Hz. The absolute steric course of the addition of the nitrogen nucleophile across the double bond of cinnamic acid was established to be *cis* since ozonolysis of (**9**) and (**14**) in formic acid, gave DL-[2-^{2}H]-aspartic acid, shown to be the *erythro*-isomer by ^{1}H n.m.r. spectroscopy and comparison with an authentic sample.[6]



(17) β-H_R = D; β-H_S = γ-H_R = γ-H_S = H
(18) β-H_S = D; β-H_R = γ-H_R = γ-H_S = H
(19) γ-H_S = D; β-H_R = β-H_S = γ-H_R = H
(20) γ-H_R = D; β-H_R = β-H_S = γ-H_S = H



(21) H_R = R = X = H; H_S = D
(22) H_S = R = X = H; H_R = D
(23) H_R = H; H_S = D; R = COMe; X = Br
(24) H_R = H; H_S = D; R = COMe; X = N_3
(25) H_R = H; H_S = D; R = COMe; X = NH_2

The above-mentioned steric course was also observed using ethyl cinnamate (**4**) as substrate. However, from (*Z*)-ethyl cinnamate (**3**) in deuteriated solvents a mixture of [(**9**) and (**14**)] and [(**10**) and (**15**)] in a ratio of *ca.* 8:2 was obtained as shown by the relative intensities of the 2-H signals, thus indicating that with the *cis*-isomer as substrate the reaction is only partially stereospecific.

Recent studies[7] on the mechanism of addition of α-nucleophiles onto αβ-unsaturated substrates have shown that the isoxazolidone (**7**) is obtained from ethyl cinnamate (**4**) and *N*-methylhydroxylamine through the possible intermediacy of the *O*-acyl derivative (**5**). In our experiments, *N*-methylhydroxylamine and both (*E*)-[α-^{2}H]cinnamic acid (**1**) and ethyl (*E*)-[α-^{2}H]cinnamate (**6**) gave the deuteriated isoxazolidone (**8**), showing n.m.r. signals due to the ring protons at δ 3·45 and 2·53 (d, J_{BX} 11·8 Hz). The latter compound was converted (H_2, Raney-nickel, followed by methylation) into 3-*NN*-dimethyl-3-phenyl[2-^{2}H]propionic acid [(**11**) and (**16**)], whose ^{1}H n.m.r. spectrum was identical to that of the compound obtained from [(**9**) and (**14**)] upon methylation. Compound (**8**) loses deuterium upon mild alkaline treatment.

Resolution[8] of [(**9**) and (**14**)] and [(**10**) and (**15**)] gave (2*R*, 3*S*)-(**9**) and (2*S*,3*S*)-(**10**), reduced in boiling dioxan with $LiAlH_4$ to the alcohols (**12**) and (**13**). Compounds (**12**)

and (**13**), after acetylation, upon ozonolysis, oxidative work up, and acid hydrolysis, gave L-(βR) [β-^{2}H]homoserine (**17**) and the (βS)-isomer (**18**), respectively.

Homoserine stereospecifically labelled in the terminal methylene group was prepared from (1*S*)-3-phenyl[1-^{2}H]-propanol[9] (**21**), which, after acetylation was brominated to (**23**), leading, in turn, to the azide (**24**). Hydrogenation of (**24**) gave the amine (**25**) which, upon ozonolysis in formic acid, afforded DL-(γS)[γ-^{2}H]homoserine (**19**). DL-(γR)-[γ-^{2}H]Homoserine (**20**) was similarly prepared from (1*R*)-3-phenyl[1-^{2}H]propanol (**22**) prepared from (**21**) by known procedures.[10]

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