Stereochemical Course of the Elimination Catalysed by L-Phenylalanine Ammonia-lyase and the Configuration of 2-Benzamidocinnamic Azlactone

By K. R. HANSON

(*Biochemistry Department, The Connecticut Agricultural Experiment Station, P.O. Box* 1106, *New Haven, Connecticut* 06504)

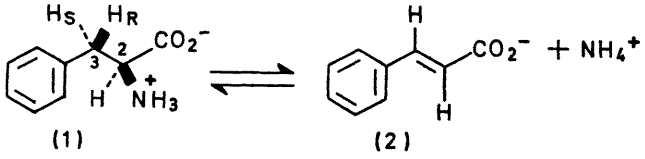
and R. H. WIGHTMAN, J. STAUNTON, and A. R. BATTERSBY*

(*University Chemical Laboratory, University of Cambridge*, CB2 1EW)

Summary Two rational syntheses of L-phenylalanine are described which allow stereospecific labelling with isotopic hydrogen at C-3; the labelled materials are used to establish that L-phenylalanine ammonia-lyase eliminates the *pro-S* proton from C-3 of L-phenylalanine together with ammonia to generate *trans*-cinnamate (*antiperiplanar* elimination).

L-PHENYLALANINE AMMONIA-LYASE[1] (EC 4.3.1.5) catalyses the elimination of a proton and ammonia from L-phenylalanine (**1**) to give *trans*-cinnamate (**2**) which is further transformed in higher plants into a vast range of phenylpropanoid derivatives.

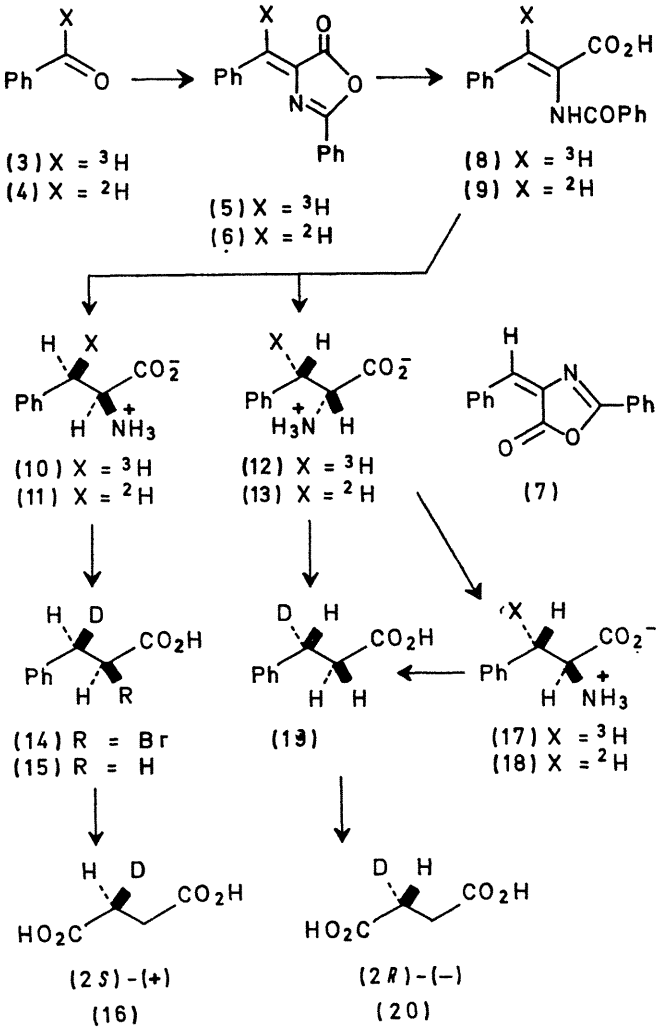
The C-3 carbon atom of phenylalanine is a prochiral centre[2] carrying two stereoheterotopic hydrogen atoms and, if the elimination reaction (**1**)→(**2**) is stereospecific, then either the *pro-R* or *pro-S* hydrogen atom will be lost [marked H_R and H_S on (**1**)]†. Two syntheses of phenylalanine bearing stereospecific labels at C-3 have been devised to determine the stereochemistry of the elimination step.



(1) (2)

For *the first synthesis*,[3] [7-^{3}H]benzaldehyde[4] (**3**) was converted into one azlactone, m.p. 165—166°, of structure (**5**), or less probably (**7**); the interpretation of the relevant n.m.r. and other data has been disputed.[5] Opening the azlactone with ethoxide, hydrogenation of the product over palladium (four separate hydrogenations were run which were expected[6] to be cleanly *syn*-stereospecific), and acidic hydrolysis of the products afforded four samples of DL-[3-$^3H_1$]phenylalanine. These were treated separately with L-phenylalanine ammonia-lyase from potatoes[7,8] until the L-isomer had been converted *completely* into cinnamate; formation of cinnamate from D-phenylalanine is negligibly slow.[7] The ^{3}H released to the aqueous medium in each of the four preparations corresponded to 0·5% of that present in the original L-phenylalanine. This result, in conjunction with those described later, shows that both the hydrogenation and the enzymatic elimination process are *at least* 99·5% *stereospecific*. The configuration at C-3 of the L-phenylalanine produced by this synthesis was established as follows (correct absolute configurations are shown throughout).

The azlactone (**6**) derived from [7-^{2}H]benzaldehyde[9] (**4**) was opened with sodium hydroxide, the resultant acid (**9**) was hydrogenated, and the products were hydrolysed by acid to the racemate (3*R*)-L-, plus (3*S*)-D-[3-$^2H_1$]phenylalanine (**11** + **13**). Conversion of (**11** + **13**) into the



(16) (20)

corresponding *N*-chloroacetyl derivatives and resolution with hog kidney acylase-I[10] gave the free L-isomer (**11**) and *N*-chloroacetyl-(3*S*)-D-[3-$^2H_1$]phenylalanine. The racemisation at C-2 during the acylation step was estimated to be ⩽10% by carrying out a parallel run in D_2O with unlabelled DL-phenylalanine and examining the product by mass spectrometry.

Nitrous acid–hydrogen bromide[11] converted (**11**) into (2*S*,3*R*)-2-bromo-3-phenyl[3-$^2H_1$]propionic acid (**14**) which was reduced catalytically to (3*S*)-3-phenyl[3-$^2H_1$]propionic

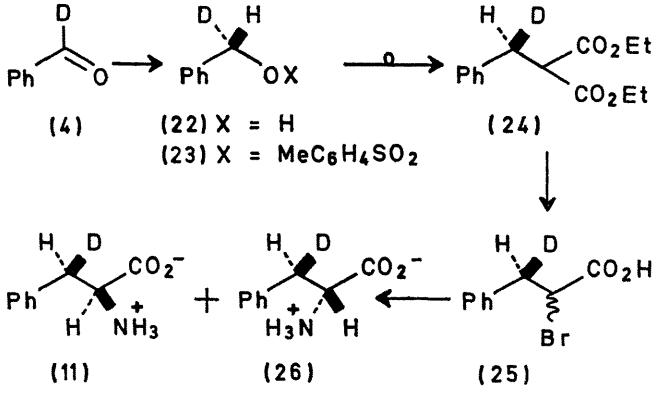
† *pro-S* and *pro-R* refer throughout to unlabelled materials. For this terminology, the application of the *R*/*S*-system to labelled compounds, and other stereochemical nomenclature see ref. 2.

acid (**15**). Ozonolysis of (**15**) and oxidation gave [2-$^2H_1$]-succinic acid shown by mass spectrometric and o.r.d. measurements[12] to contain 81 ± 8% of the (2-*S*)-isomer (**16**). Complementary evidence was gained by racemising with acetic anhydride the C-2 centre of (**13**) and degrading the product (**13** + **18**) *via* (**19**) to [2-$^2H_1$]succinic acid: 77 ± 8% of (2*R*)-isomer (**20**).

These results establish the configuration at C-3 of the [3-$^2H_1$]phenylalanines and, if *syn* addition of hydrogen is accepted, they also show that the Plöchl–Erlenmeyer azlactone has the *Z*-configuration (**6**), *i.e.*, the bulky substituents are *trans*.

As the tritiated L-phenylalanines produced directly by the above synthesis must have had the *R*-configuration at C-3, the foregoing enzymatic experiments on the tritiated substrate prove the specific loss of the *pro-S* hydrogen atom in the elimination step.

This was confirmed by work with the $^2H_1$-labelled phenylalanines. On complete enzymatic conversion of the (3*R*)-L-isomer of (**11** + **13**) into cinnamate, mass spectrometry supported by n.m.r. showed 98 ± 4% ^2H retention, whereas the (3*S*)-L-isomer of (**18** + **13**) gave 0 ± 4% ^2H retention.



The elimination process at pH 8·7 is subject to a statistically significant primary ^3H isotope effect which was demonstrated by observing the degree of retention of ^3H in (3*S*)-L-[3-$^3H_1$]phenylalanine when the enzymatic reaction was *ca.* 45% complete. [1-^{14}C]Phenylalanine was used as internal reference and the observed rise in ^3H:^{14}C ratio from 5·90 at the outset to 6·97 in the recovered L-phenylalanine corresponds to a discrimination factor against ^3H of at least 1·2. This value will be lower than the true kinetic isotope effect because of (a) the presence of 11% of the (3*R*)-isomer in the sample used causing enhanced rate of loss of ^3H from the phenylalanine pool and (b) possible enzyme-mediated exchange of ^3H from the (3-*S*)-isomer without formation of cinnamate. A parallel experiment with (3*R*)-L-[3-$^3H_1$]-phenylalanine showed an increase in ratio from starting material to recovered amino-acid only of 6·05 to 6·21 which, if significant, could be attributed to the 11% of (3*S*)-isomer present in the sample.

The *second synthesis* used liver alcohol dehydrogenase to introduce the asymmetry providing a link between two important networks of configurationally related materials. Enzymatic reduction of [7-$^2H_1$]benzaldehyde (**4**) afforded (1*S*)-[1-$^2H_1$]benzyl alcohol[13] (**22**) which as the corresponding toluene-*p*-sulphonyl derivative (**23**) was treated with sodiomalonic ester to yield (**24**). Hydrolysis followed by bromination gave (**25**) which was then converted by ammonia into equimolar amounts of (3*R*)-D- and (3*R*)-L-[3-$^2H_1$]phenylalanine (**11** + **26**); found 96 ± 4% $^2H_1$. Degradation of (**24**) *via* (**15**) afforded (**16**): 95 ± 5% of (2*S*)-[2-$^2H_1$]succinic acid. L-Phenylalanine ammonia-lyase converted the L-isomer (**11**) of (**11** + **26**) into [3-$^2H_1$]cinnamic acid: found 98 ± 4% $^2H_1$, proving, like the foregoing results, that enzyme specifically eliminates the *pro*-3*S* hydrogen atom of L-phenylalanine.

The elimination reaction is not accompanied by a significant loss or exchange of the hydrogen at C-2. When DL-[2-$^3H_1$]phenylalanine[14] was treated with the enzyme and the reaction was taken to completion, less than 1% of the tritium in the L-enantiomer was released.

The rigorously defined stereochemistry of the enzymatic elimination process thus corresponds to that catalysed by L-histidine ammonia-lyase[15] with a mechanism in which the *pro-S* hydrogen and the product of the reaction between the substrate $-NH_3^+$ and the enzyme's prosthetic group[8] are eliminated in an *antiperiplanar* manner to yield *trans*-cinnamate directly.

We thank Professor J. W. Cornforth, Dr. D. R. Robinson, and Dr. G. Ryback (Sittingbourne) for the o.r.d. measurements on deuteriated succinic acids here and for the preceding communication, Dr. Evelyn Havir for collaboration in the enzymatic studies, and the following for financial support: New Haven, The National Science Foundation; Cambridge, The Nuffield Foundation, and the S.R.C.

(*Received, December 9th*, 1970; *Com.* 2128.)

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