# Steric Course and Rearrangements in the Biosynthesis of Phenylalanine, Tyrosine, and 3-(3-Carboxyphenyl)alanine from Shikimic Acid in Higher Plants

By P. OLESEN LARSEN*

(*Organic Chemical Laboratory, Royal Veterinary and Agricultural University, Copenhagen, Denmark*)

and D. K. ONDERKA and H. G. FLOSS

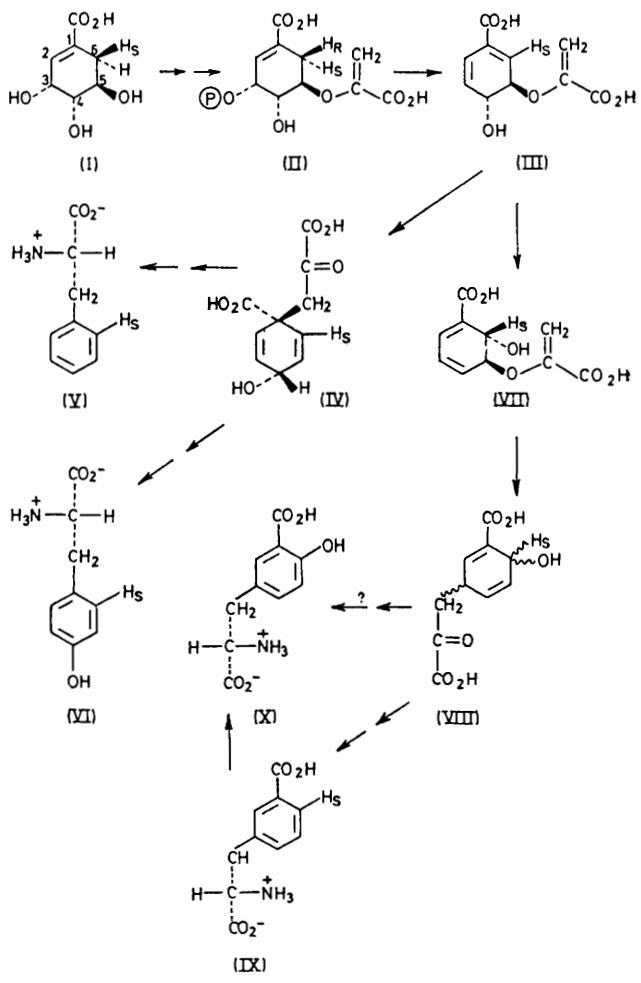
(*Department of Medicinal Chemistry and Pharmacognosy, Purdue University, Lafayette, Indiana* 47907)

*Summary* Incorporation of stereospecifically labelled shikimic acid into *Reseda lutea* L. demonstrates the same steric course and rearrangement in the biosynthesis of phenylalanine and tyrosine as that known from bacteria, whereas the biosynthesis of 3-(3-carboxyphenyl)alanine involves a different rearrangement reaction.

IN micro-organisms, phenylalanine (V) and tyrosine (VI) are synthesised from shikimic acid (I) *via* chorismic acid (III) and prephenic acid (IV) by a well known pathway.[1] The same pathway is presumed to operate in higher plants. This conclusion is partly based on the incorporation, in higher plants, of (I) into a variety of aromatic compounds,[2] and of (III) into (V) and (VI),[3] and partly on the identification of some of the necessary enzymes in plants.[2] We now present evidence which further supports this conclusion. Concurrently, evidence is presented indicating that the *m*-carboxy-substituted aromatic amino-acids, present in various higher plants and known to be derivatives of (I),[4] are derived from (III) through special rearrangements not involving (IV).

Experiments were performed with *Reseda lutea* L., known to contain 3-(3-carboxyphenyl)alanine (IX) and 3-(3-carboxy-4-hydroxyphenyl)alanine (X) [together with very small amounts of (3-carboxyphenyl)glycine and (3-carboxy-4-hydroxyphenyl)glycine].[4] Labelled compounds were fed to Reseda leaves, the amino-acids isolated, and the incorporation percentage determined by methods previously described.[4] Compounds (V) and (VI) were isolated from the free neutral amino-acid fraction by use of a Sephadex column.[5] The Table shows the results obtained by incorporation of double-labelled (I) carrying a stereospecific tritium label at C-6.[6] [$^{14}$C]Glucose was used for comparison.

The tritium incorporated into (V) and (VI) was shown to be located in one of the positions *ortho* to the side-chain. Compound (V) was degraded *via* benzoic acid and aniline to



*p*-bromoacetanilide (100% tritium retention) and tribromoaniline (10% tritium retention) by methods described in the literature.[7] Compound (VI) was transformed into 3',5'-dibromotyrosine[8] (85% tritium retention) and by ozonisation into aspartic acid[9] (2% tritium retention).

The tritium incorporated into (IX) was located in a position *para* to the $C_3$ side-chain. Transformation *via m*-(2-aminoethyl)benzoic acid into 2-phenylethylamine was performed as previously described.[4] Permanganate oxidation of 2-phenylethylamine yielded benzoic acid, which was transformed *via* aniline into *p*-bromoacetanilide by standard methods (93% tritium retention in acetanilide, 0% tritium retention in *p*-bromoacetanilide).

TABLE

*Incorporation of labelled precursors into leaves of* Reseda lutea *L.*

| Precursor | | (6*R*)-Shikimic acid U-$^{14}$C-6-$^{3}$H[a] | (6*S*)-Shikimic acid U-$^{14}$C-6-$^{3}$H[a] | Glucose U-$^{14}$C |
|---|---|---|---|---|
| $\mu$Ci $^{14}$C fed | | 5·7 | 2·7 | 5·0 |
| $^{3}$H/$^{14}$C | | 4·6 | 4·0 | — |
| Phenylalanine | % Inc. | 0·62 | 0·78 | 0·076 |
| | $^{3}$H/$^{14}$C | 0·2 | 3·9 | — |
| | % T-ret.[b] | 4[c] | 84[c] | — |
| Tyrosine | % Inc. | 0·14 | 0·10 | 0·011 |
| | $^{3}$H/$^{14}$C | 0·2 | 4·1 | — |
| | % T-ret.[b] | 4[c] | 88[c] | — |
| 3-(3-Carboxyphenyl)alanine | % Inc. | 0·55 | 0·14 | 0·010 |
| | $^{3}$H/$^{14}$C | 0·3 | 4·7 | — |
| | % T-ret.[b] | 7 | 118 | — |
| 3-(3-Carboxy-4-hydroxyphenyl)alanine | % Inc. | 0·13 | 0·053 | 0·0027 |
| | $^{3}$H/$^{14}$C | 0 | 0 | — |
| | % T-ret.[b] | 0 | 0 | — |

[a] The precursor contained 85—90% of the tritium as indicated and 10—15% in the diastereotopic position (*cf.* ref. 6).
[b] % Tritium retention = 100 × ($^{3}$H/$^{14}$C of product)/($^{3}$H/$^{14}$C of precursor).
[c] Corrected for loss of 1/7th of $^{14}$C.

The *pro*-6*S*-hydrogen atom is retained and the *pro*-6*R*-hydrogen atom is lost in the enzymatic transformation of (II) into (III) using *Aerobacter aerogenes* or *Escherichia coli* as source of the enzyme.[6,10] The present results indicate that the steric course of this reaction is the same in higher plants.

The location of the tritium in the *ortho*-position in (V) and (VI) is that expected when the $C_3$ side-chain is attached to C-1 in the ring from (I). This direct evidence for the rearrangement from (III) to (IV) in higher plants is new, although results obtained by incorporation of (I) labelled specifically with $^{14}$C into lignin with subsequent degradation and location of the radioactivity may be interpreted in the same way.[11]

The *pro*-6*S*-hydrogen atom of (I) is also retained in (IX), supporting the view that this amino-acid is derived from (I) *via* (II) and (III). The location of the tritium atom at the *para*-position of (IX) demonstrates that the $C_3$ side-chain in this amino-acid is attached to the original C-3 of shikimic acid. A possible pathway would be rearrangement of (III) into isochorismic acid (VII), rearrangement of (VII) into isoprephenic acid (VIII), loss of water in (VIII) to give 3-(3-carboxyphenyl)pyruvic acid, and transamination to (IX). Compound (VII) has previously been identified in *Aerobacter aerogenes* and chemical rearrangement to 3-(3-carboxyphenyl)pyruvic acid has been observed.[12] Rearrangement of (III) into 3-(3-carboxy-6-hydroxycyclohexa-2,4-dienyl)pyruvic acid with subsequent dehydration to give 3-(3-carboxyphenyl)pyruvic acid is unlikely, since this rearrangement would be forbidden as a thermal process according to the Woodward–Hoffman rules.[13] The rearrangements of (III) into (IV) and of (VII) into (VIII) are thermally allowed processes.

Compound (X) may be derived from (VIII) by oxidation and transamination. This would account for the loss of tritium. Direct hydroxylation of (IX) into (X) has, however, been observed.[14] If the NIH shift is operating, this would result in tritium labelling of (X) in the 5'-position. Loss of tritium in this *ortho*-position to a phenolic group through exchange during isolation can, however, not be completely excluded.

Support from the Danish Natural Science Research Council (to P.O.L.) and from the U.S. Public Health Service (to H.G.F.) is acknowledged.

(*Received, 17th April* 1972; *Com.* 657.)

[1] F. Lingens, *Angew. Chem. Internat. Edn.*, 1968, **7**, 350.
[2] S. Yoshida, *Ann. Rev. Plant Physiol.*, 1969, **20**, 41.
[3] E. Leistner and M. H. Zenk, *Z. Naturforsch.*, 1968, **23b**, 259.
[4] P. O. Larsen, *Biochim. Biophys. Acta*, 1967, **141**, 27.
[5] B. Kowalska, *Acta Biochim. Polon.*, 1964, **16**, 141.
[6] D. K. Onderka and H. G. Floss, *J. Amer. Chem. Soc.*, 1969, **91**, 5894; H. G. Floss, D. K. Onderka, and M. Caroll, *J. Biol. Chem.*, 1972, **247**, 736.
[7] C. Gilvarg and K. Bloch, *J. Biol. Chem.*, 1952, **199**, 689.
[8] R. Zeynek, *Z. physiol. Chem.*, 1921, **114**, 275.
[9] A. Previero, E. Scoffone, P. Pajetta, and C. A. Benassi, *Gazzetta*, 1963, **93**, 841.
[10] R. K. Hill and G. R. Newkome, *J. Amer. Chem. Soc.*, 1969, **91**, 5893.
[11] G. Eberhardt and W. J. Schubert, *J. Amer. Chem. Soc.*, 1956, **78**, 2835.
[12] I. G. Young, T. J. Batterham, and F. Gibson, *Biochim. Biophys. Acta*, 1969, **177**, 389.
[13] G. B. Gill, *Quart. Rev.*, 1968, **22**, 338.
[14] P. O. Larsen and H. Sørensen, *Biochim. Biophys. Acta*, 1968, **156**, 190.