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STERIC COURSE OF THE TYROSINE AMMONIA-LYASE REACTION

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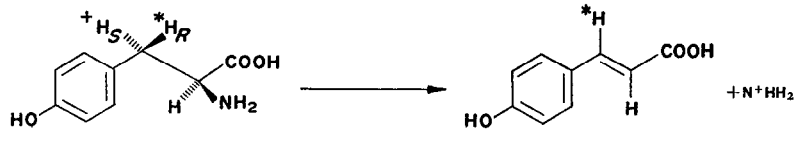
Abstract—The tyrosine ammonia-lyase reaction proceeds with loss of the *pro*-3*S* and retention of the *pro*-3*R* hydrogen from the tyrosine side chain and thus involves *anti*-periplanar elimination of the elements of ammonia.

TYROSINE ammonia-lyase is an enzyme present in a variety of higher plants,[1,2] primarily Gramineae, and in some fungi,[3] which catalyses the elimination of the elements of ammonia from *S*-tyrosine (I) to give *trans*-*p*-coumaric acid (II). In connection with studies on another enzyme, tyrosine phenol-lyase, we determined the steric course of this reaction using tyrosine ammonia-lyase from young maize cobs isolated according to Neish.[1] (2*S*,3*R*)- and (2*S*,3*S*)-tyrosine-3-*T*, which served as substrates, had been prepared earlier by *cis*-hydrogenation of appropriately tritiated α-acylaminocinnamic acids and their configuration had been



(I) (II)

determined by degradation to aspartic acid of known stereochemistry.[4] These two samples were each mixed with (2*S*)-tyrosine-U-^{14}C to give $T/^{14}C$ ratios of 10·25 and 9·9 respectively, and then incubated with 6·5 Units[1] enzyme and 100 μmol K_2BO_3 (pH 8·8) in a total vol. of 0·7 ml at 40° for 4 hr. *p*-Coumaric acid (87 and 90% yield, respec.) as well as unreacted tyrosine were isolated from the incubation mixtures, purified by chromatography (TLC, PC) and their $T/^{14}C$ ratios were determined. In the experiment using the 3*R* isomer the *p*-coumaric acid had $T/^{14}C = 8{\cdot}63$ and the recovered tyrosine $T/^{14}C = 9{\cdot}8$; in the experiment with the 3*S* isomer the figures were 1·36 and 9·07. The average tritium retentions of

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85·1% from (2*S*,3*R*)-tyrosine-3*T* and 14·4% from (2*S*,3*S*)-tyrosine-3-*T* clearly indicate loss of the *pro*-3*S* and retention of the *pro*-3*R* hydrogen from the tyrosine side chain. This finding is in agreement with results from the laboratories of Battersby and Hanson[5] and shows that the tyrosine ammonia-lyase reaction apparently involves an *anti*-periplanar elimination of the elements of ammonia. The stereochemistry of this reaction thus conforms to that of the other ammonia-lyase reactions studied, i.e. aspartase,[6] methyl-aspartase,[7] histidine ammonia-lyase[8] and phenylalanine ammonia-lyase.[9] The incomplete loss and retention of tritium from the two samples in the above experiments in all likelihood reflects incomplete stereochemical purity of the substrates rather than incomplete stereospecificity of the enzyme towards the diastereotopic hydrogens at the methylene group of tyrosine. This is suggested very strongly by the fact that in earlier experiments almost identical tritium retention values had been observed during the conversion of these tyrosines into haemanthamine.[4]

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