Stereochemistry of the Enzymatic and Non-enzymatic Conversion of 3-Dehydroshikimate into Protocatechuate

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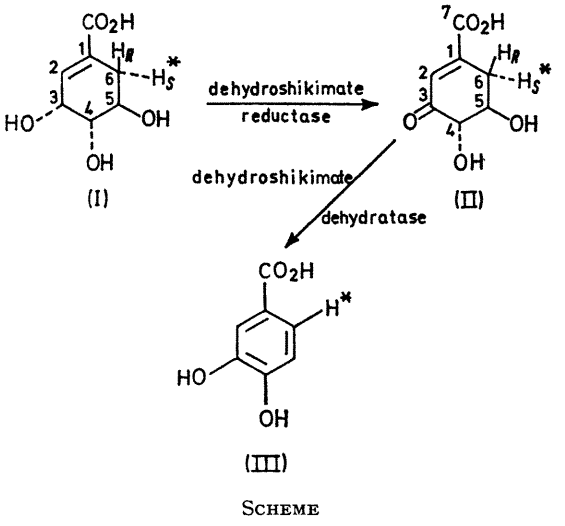
Summary Using substrate tritiated stereospecifically at C-6 it was shown that the dehydroshikimate dehydratase reaction involves the *syn*-elimination of the elements of water, whereas the acid-catalysed chemical conversion of 3-dehydroshikimate into protocatechuate proceeds non-stereospecifically and involves a hydrogen isotope effect.

THE formation of aromatic compounds by the shikimate pathway usually proceeds through chorismic acid.[1] However, there are some exceptions to this rule, most notably the conversion of dehydroshikimic acid (II) into protocatechuic acid (III), which was first observed in a mutant of *Neurospora crassa*,[2] but also occurs in other micro-organisms.[3,4] Dehydroshikimate dehydratase, the enzyme catalysing this conversion, is very heat labile and has therefore not been studied extensively.[5] Isotope data showed that the reaction involves loss of the hydroxy-group at C-5,[6] suggesting that the enzyme catalyses the elimination of water from C-5 and C-6 of the enol form of dehydroshikimate.[5] We have now determined the steric course of this elimination with respect to the hydrogens at C-6. (6*R*)- and (6*S*)-[6-^{3}H]shikimic acid (I), each containing 80—90% of the label in the position specified and the remainder in the diastereotopic hydrogen at C-6, were prepared as indicated previously[7] and were mixed with [7-^{14}C]shikimic acid obtained by the same enzymatic route from [1-^{14}C]phosphoenolpyruvate.[8] The two samples of shikimate (*ca.* 0·1 μmol) were then converted into 3-dehydroshikimates[5] using a dialysed cell-free extract of *Aerobacter aerogenes* 62-1 (81 and 79% yield). After chromatographic purification, these were converted into protocatechuate by incubation with a cell-free extract of *N. crassa* mutant arom-1 for 2·5 h at 30° in a nitrogen atmosphere as described by Gross.[5] The protocatechuate samples (*ca.* 60%

Enzymatic and non-enzymatic conversion of (6R)- *and* (6S)-[6-^{3}H,7-^{14}C]-3-*dehydroshikimate into protocatechuate*

Compound	(6*R*)-[6-^{3}H,7-^{14}C]-Shikimate	(6*S*)[6-^{3}H,7-^{14}C]-Shikimate
Enzymatic conversion		
^{3}H:^{14}C of shikimate (*ca.* 1·25 μCi ^{14}C):	6·66	5·13
3-dehydroshikimate:	6·40	5·38
protocatechuate:	0·96 ± 0·16	4·92 ± 0·29
^{3}H-retention (II) → (III):	15·0	91·5
Non-enzymatic conversion		
^{3}H:^{14}C of 3-dehydroshikimate:	2·42	1·98
protocatechuate:	2·08 ± 0·06	1·73 ± 0·09
^{3}H-retention (II) → (III):	86	87



SCHEME

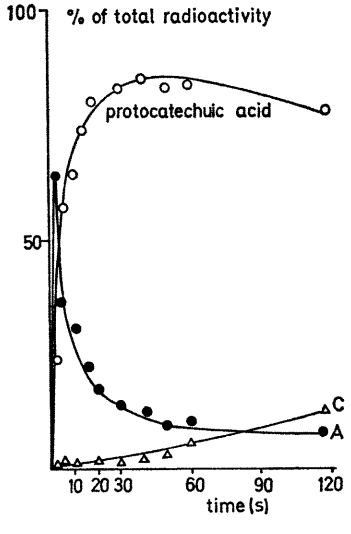


FIGURE. *Transformation of* 3-*dehydroshikimic acid in conc.* HCl *at* 98—99°.

yield) were purified by paper and thin layer chromatography as such, after methylation with CH_2N_2 as veratric acid methyl ester and after alkaline hydrolysis of the ester as veratric acid, and the $^3H:^{14}C$ ratios were measured after each purification step. The results (see Table) show that in the 3-dehydroshikimate dehydratase reaction the *pro*-6*R* hydrogen is eliminated together with the hydroxy-group at C-5 (Scheme). The reaction thus involves the rather unusual *syn*-elimination of the elements of water, which has before only been observed in the dehydroquinate dehydratase reaction.[9] We have also investigated the chemical conversion of 3-dehydroshikimic acid into protocatechuic acid, which takes place upon pyrolysis[10,11] or by heating with conc. HCl in a bath of boiling water.[11] Under the latter conditions the reaction proceeds through a rapidly formed intermediate (A, Figure) which is transformed into protocatechuate. This is slowly decomposed to an unknown product C. The protocatechuic acids isolated after 60 s from reaction mixtures containing (6*R*)- or (6*S*)-[6-^{3}H,7-^{14}C]-3-dehydroshikimic acids had both retained the same amount of tritium relative to ^{14}C, 86 and 87%, respectively (Table), indicating that the chemical conversion is non-stereospecific and involves a large isotope effect. Although the data do not necessarily prove that elimination of the *pro*-6*R* and the *pro*-6*S* hydrogen proceed at the same rate, both must obviously involve equally large isotope effects, suggesting that they occur by the same mechanism, presumably a non-concerted one.†

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† As suggested by a referee, the reaction may proceed through the 4α,5α-epoxide.

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