The Stereochemical Course of the 3-Dehydroquinate† Dehydratase Reaction and a Novel Preparation of Shikimic Acid Labelled with Isotopic Hydrogen at C-2

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Summary A preparation of shikimic acid labelled with isotopic hydrogen at position 2 is described, and the *syn* stereochemical course of the 3-dehydroquinate dehydratase reaction is confirmed.

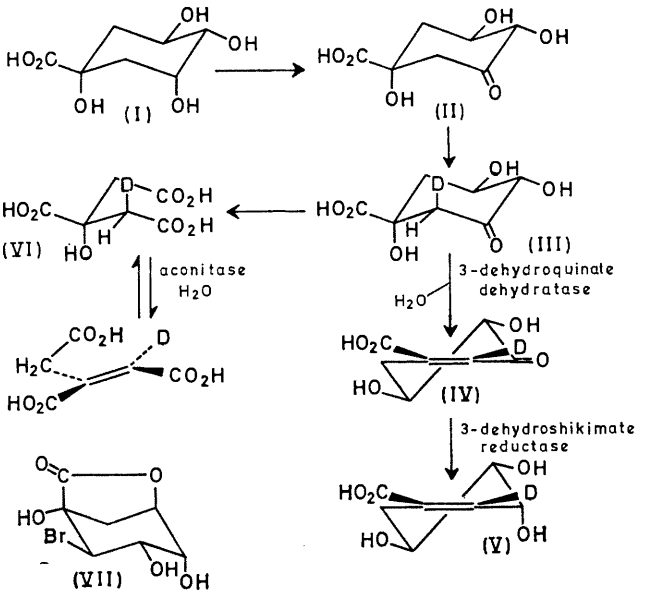
IN the course of studies of phenol metabolism in plants it became necessary to prepare shikimic acid labelled isotopically at the 2-position. Stereospecific catalytic oxidation of (−)-quinic acid† (I) gave 3-dehydroquinic acid (II),[1,2] the first cyclic intermediate in the shikimate pathway.[3] The ^1H n.m.r. spectrum of (II) in D_2O was in agreement with the adoption of the conformation (II) by both the anion and the free acid in aqueous solution. The methylene protons at C-2 were distinct as two doublets (J 14 Hz) and a novel feature was the appearance at lower chemical shift of 2 eq-H, τ 6·92 in acid, compared to 2ax-H, τ 6·35. This is a further example[4] of the reversal of the normal rule as stated by Lemieux.[5] In D_2O solution at pH 8·5 the three protons at C-2 and C-4 were exchanged for deuterium. At pH 7·0 the exchange was site and stereoselective (*ca.* 90%) and provided a means of preparing 2(*S*)-deuterio-3-dehydroquinic acid (III) with 0·6—0·7 atom deuterium incorporation. A mechanism for catalysis of the exchange of H_S (2 axial) in preference to H_R(2 equatorial) and 4-H may be written involving the carboxylate ion of (II). Proof of the stereochemical assignment of the deuterium configuration in (III) and hence of the ^1H n.m.r. analysis of (II) was derived by degradation ($NaIO_4$, Br_2 water) to deuteriocitric acid (VI). The citric acid was equilibrated (24 hr.) with pigs-heart aconitase[6] in both D_2O and H_2O and the acid re-isolated, converted into its trimethyl ester, and examined by mass spectrometry. The deuterium was resistant to enzymic exchange in H_2O, and in D_2O the citric acid incorporated a further 0·6 atom of deuterium.

From the known stereochemical features of the aconitase reaction[7,8] it was possible to infer that the deuterium had the *S* configuration in the *pro-R*-methylene group of citric acid (VI).

Catalytic reduction of 2(*R*)-bromoquinide (VII)[9] with deuterium gas gave 2(*RS*)-deuteriouquinide which was converted into the acid and thence by catalytic oxidation to 2(*RS*)-deuterio-3-dehydroquinic acid. The ^{1}H n.m.r. spectrum of the acid showed 0·7 atom deuterium with the 2(*R*)-configuration and 0·3 atom deuterium with the 2(*S*)-configuration thus indicating that the original hydrogenolysis of (VII) proceeded with predominant inversion at C-2. Equilibration of the acid with H_2O at pH 7·0 gave 2(*R*)-deuterio-3-dehydroquinic acid, with 0·65 atom of deuterium, and proof of structure followed the pattern established for (III). Both diastereoisomers may also be prepared by analogous procedures using tritium rather than deuterium as the isotopic label.

Treatment of the diastereoisomeric deuterio-acids with an enzyme extract from *Escherichia coli* 83-24 coupled with the cyclic generation of NADPH from NADP by isocitrate dehydrogenase[10] and isocitrate gave samples of shikimic acid which were examined by ^{1}H n.m.r. spectroscopy, and, as the methyl triacetate,[11] by mass spectroscopy. Loss of deuterium was observed with the 2(*R*)-deuterio-acid. Shikimic acid labelled with deuterium at the 2 position (V; 0·6 atom deuterium) was formed from (III). Shikimic acid similarly labelled (V; 0·3 atom deuterium) was prepared by similar means from 2(*RS*)-deuterio-3-dehydroquinic acid.

These results provide a novel procedure for the preparation of shikimic acid isotopically labelled at position 2. They also confirm that the 3-dehydroquinate dehydratase reaction follows the unusual course of a *syn*-elimination[12] and thus complement the earlier observations of Hanson and Rose[8] on the reverse hydration reaction.



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† Nomenclature of quinic and shikimic acids. The IUPAC–IUB nomenclature rules for cyclitols (*European J. Biochem.*, 1968, **5**, 1—12) indicates that the numeration of these systems should be revised as shown. The biochemical intermediates 5-dehydroquinic and 5-dehydroshikimic acids thus become 3-dehydro-acids.

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