terminal hydrogens lie approximately on an extension of the line joining each boron with the center of the icosahedron of which the boron framework may be imagined to be a part. The bridge hydrogens lie approximately in the surface of the same icosahedron and below the base borons. The rupture of one end of a hydrogen-bridge bond gives a hydrogen quite different from the terminal hydrogen already present. Only the "new terminal hydrogen" is in a position to re-form as a bridge, either in its previous position or over what was previously the short boron–boron bond. This provides a consistent explanation of both the products of the exchange reaction with diborane(6) and the nmr spectrum. Both the intermolecular and intramolecular exchange reactions described here are of types not previously observed for boron hydrides.

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(10) National Aeronautics and Space Administration Predoctoral Trainee.

James C. Carter, Nancy L. H. Mock[10]
Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15213
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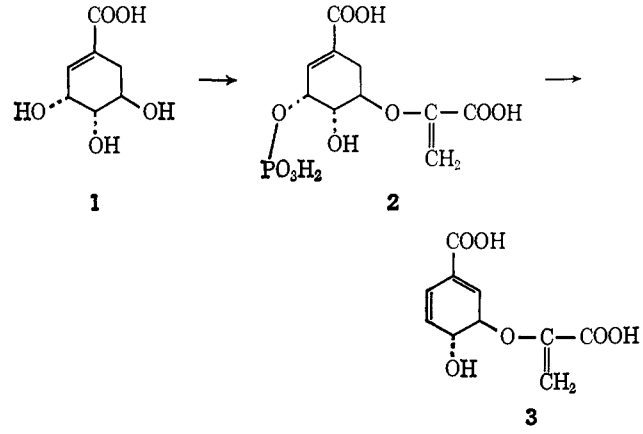
Stereochemistry of Chorismic Acid Biosynthesis[1]

Sir:

The pathway of aromatic ring biosynthesis in bacteria from glucose *via* shikimic acid (**1**) has been elucidated in considerable detail.[2] In this sequence chorismic acid (**3**) plays a central role as branch point,[3] leading to many important groups of aromatic natural products: the aromatic amino acids (phenylalanine, tyrosine, and tryptophan), anthranilic acid and (in some organisms) nicotinic acid, *p*-aminobenzoic acid and folic acid, *p*-hydroxybenzoic acid and ubiquinone, vitamin K_2, and salicylic acid and its hydroxylated derivatives.[4]

The steps from shikimic acid to chorismic acid have been clarified by Sprinson and coworkers,[5] who showed that shikimate 5-phosphate is converted to the 3-enolpyruvate **2**, which then undergoes 1,4 elimination of phosphoric acid. This elimination, mediated by chorismate synthetase, presents an interesting stereochemical question with which this communication deals: does such a 1,4-conjugate elimination show a preference for proceeding *cis* or *trans?* Theory[6] predicts that a concerted 1,4-conjugate elimination should be *cis*, but studies in nonenzymatic systems have led to conflicting conclusions.[7] By using stereospecifically deuterated shikimic acids as substrates, we have now been able to show that the chorismate synthetase reaction is a stereospecific *trans*-1,4 elimination.



Our approach to this problem was to prepare samples of shikimic acid in which each of the hydrogens at C-6 was in turn replaced by deuterium. The Diels–Alder route[8] to shikimic acid was followed to ensure stereospecific introduction of the label. Addition of *trans,trans*-1,4-diacetoxybutadiene to methyl α,*trans*-β-dideuterioacrylate[9] (**4**) (98% of two deuteriums by nmr analysis) afforded adduct[10] **5**, which was converted by the published procedure[8] to 6α-deuterioshikimic acid (**6**α-*d*). In a similar fashion, methyl *cis*-β-deuterioacrylate[9] (**6**) was converted through adduct **7** to 6β-deuterioshikimic acid (**6**β-*d*) containing 85% of one deuterium. All synthetic intermediates were characterized by ir, nmr, and mass spectroscopy, and had melting or boiling points identical with the published values. The deuterated shikimic acids were pure, recrystallized products.

Incorporation experiments were conducted with *E. coli* mutant 156-53M31, doubly blocked both immediately before and after dehydroshikimic acid, to minimize the possibility of back mutation.[11] The mutant was grown in medium A with 0.5% glucose supplemented by deuterated or natural shikimate. Growth rates on **6**α-*d* and **6**β-*d* showed that both racemic deuterated shikimic acids supported growth to exactly one-half the extent of natural (−)-shikimate. Cells were harvested after 12 hr, denatured with trichloroacetic acid, and hydrolyzed with acid. Phenylalanine and tyrosine were isolated from the hydrolysate by the method of Partridge;[12] tyrosine was then purified by recrystallization, while phenylalanine was converted to its ethyl ester and purified by preparative vpc.

(1) This investigation was supported in part by a research grant (GM-06568) from the Public Health Service, to whom we express our appreciation.

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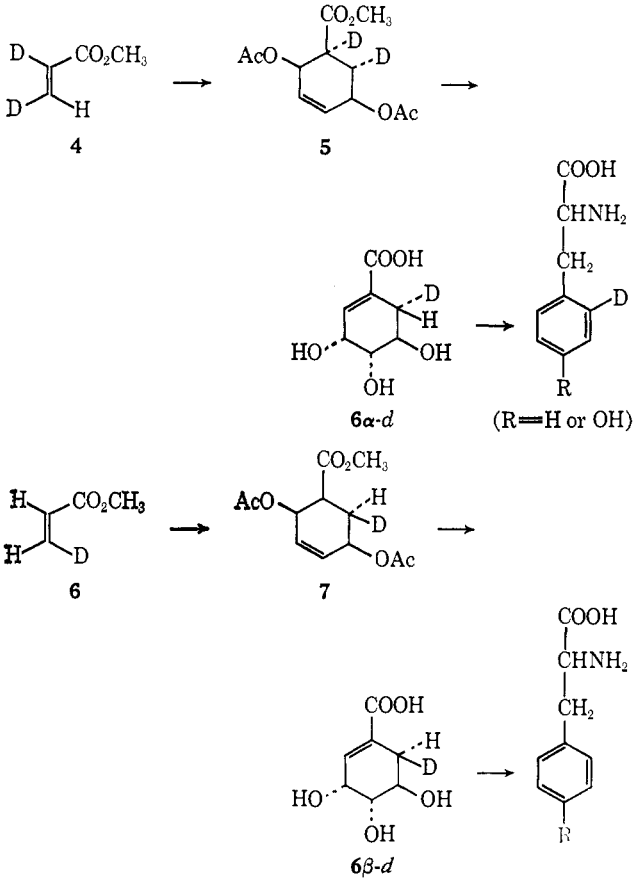
Assay of deuterium content by mass spectroscopy showed that the tyrosine and phenylalanine biosynthesized from 6α-*d* retained at least 98% of the deuterium of the precursor, while the aromatic amino acids originating from 6β-*d* were devoid of deuterium. The chorismate synthetase reaction is thus a stereospecific *trans*-1,4 elimination. Independent studies by Onderka and Floss[13] using a different approach have led to the same conclusion. These results suggest that this enzymatic elimination is probably not a concerted E2′ reaction.



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(14) Address correspondence to this author at Department of Chemistry, University of Georgia, Athens, Ga. 30601.

(15) National Institutes of Health Postdoctoral Fellow, 1967–1968.

Richard K. Hill,[14] George R. Newkome[15]
Department of Chemistry, Princeton University
Princeton, New Jersey 08540
Received June 19, 1969

Steric Course of the Chorismate Synthetase Reaction and the 3-Deoxy-D-*arabino*-heptulosonate 7-Phosphate (DAHP) Synthetase Reaction

Sir:

The reactions of the shikimic acid pathway,[1] the major route leading to the formation of aromatic compounds in plants and microorganisms, pose a number of interesting stereochemical questions. We report here data which establish the steric course of two of the reactions of the pathway, the chorismate synthetase reaction and the DAHP synthetase reaction. The formation of chorismate (VI) involves a 1,4-conjugate elimination of phosphoric acid (Scheme I) which could be either a *cis* or a *trans* process, *i.e.*, either the *pro-R* (*trans*) or the *pro-S* hydrogen (*cis*) could be removed from C-6. In the formation of DAHP (IV) from erythrose 4-phosphate (III) and phosphoenolpyruvate (II) we ask the question: from which side of the plane of the double bond is C-3 of phosphoenolpyruvate attacked?

3-Phosphoglyceric-3-*t* acids (I) 3*R* and 3*S* were prepared from mannose-1-*t* and glucose-1-*t* using the glycolytic enzymes including phosphomannose isomerase or phosphoglucose isomerase, respectively, in the presence of arsenate. These phosphoglyceric acids upon incubation with phosphoglyceromutase and enolase give the two isomeric specimens of phosphoenolpyruvate tritiated asymmetrically at C-3. Preliminary evidence[2] indicates that the enolase reaction involves a *trans* elimination of the elements of water, *i.e.*, the phosphoenolpyruvate from (3*R*)-phosphoglycerate-3-*t* would have *E* configuration[3] (phosphate and tritium *trans* to each other). The formation of phosphoenolpyruvate was coupled to its further conversion, with nonlabeled erythrose 4-phosphate, into shikimate (V) using a cell-free extract of *E. coli* mutant 83-24. The two specimens of shikimic-6-*t* acid were chromatographically purified to radiochemical homogeneity. Aliquots were mixed with shikimic-7-^{14}C acid (total 1 μmol) and converted into chorismic acids using cell-free extracts of *Aerobacter aerogenes* mutant 62-1, and the changes in the T/^{14}C ratios were determined. By enzymatic conversion into anthranilate it was ascertained that all the remaining tritium of the chorismate samples was confined to the 6 position. (*R*,*S*)-Shikimic-1,6-^{14}C acid was added to another aliquot of the tritiated shikimic acids. These samples (0.75 μmol, 1.2 μCi of ^{14}C/μmol) were esterified and the methyl esters were ozonized, followed immediately by reduction with $NaBH_4$. The products were further degraded by periodate and bromine oxidation[4] to give (*R*,*S*)-malates, which after paper chromatographic purification were subjected to the fumarase reaction. Fumarate and malate were separated by tlc. There was a slight decrease in the T/^{14}C ratios (12%) during the degradation of the shikimates to malates, presumably due to some exchange by enolization during the work-up at the aldehyde stage.

The data from these experiments are summarized in Table I. Clearly, the two shikimic acids obtained from (3*R*)- and (3*S*)-phosphoglycerate-3-*t* are predominantly stereospecifically labeled at C-6, as observed earlier,[5] although there is some scrambling of the tritium. The hydrogen at C-6 of shikimate, which originates from the *pro*-3*S* hydrogen of phosphoglycerate, is the one that is eliminated in the chorismate synthetase reaction. This hydrogen is also the one which is eliminated in the con-

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