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1,4-Radical Elimination in Cyclohexene Systems: A Model for the Chorismate Synthase Reaction

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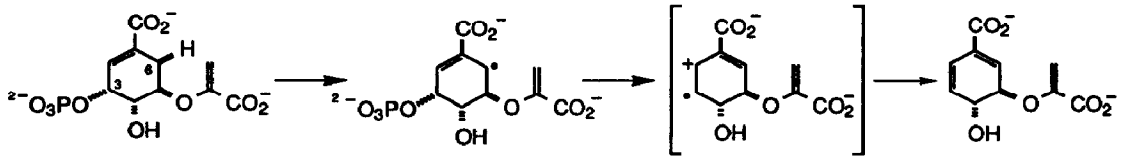
Abstract: *Treatment of cyclohexenyl bromide 9 with low concentration of Bu_3SnH gives cyclohexadiene 10 via allylic radical 14 as intermediate. This 1,4 radical elimination is a model reaction of the conversion of shikimate 1 to chorismate 4. Kinetic studies show that the elimination step from allyl radical 14 to cyclohexadiene 10 is relatively slow.*

The shikimic acid pathway is used by plants and microorganisms to biosynthesize the aromatic amino acids phenylalanine, tryptophan, and tyrosine as well as many other primary and secondary metabolites from glucose.[1] The seventh enzyme of the shikimate pathway, chorismate synthase, catalyzes the conversion of 5-enolpyruvylshikimate 3-phosphate (**1**, EPSP) to chorismate **4**.[2] The reaction involves removal of the C-6 *pro-R* hydrogen and loss of phosphate to generate a diene in what is formally a *trans* 1,4-elimination.[3] Both theoretical[4] and experimental[5] models are known to favor *cis* elimination for concerted reactions of 1,4-substituted cyclohexene systems. Therefore, several alternative proposals have been forwarded to describe the mechanism of action of chorismate synthase which include a two stage mechanism where an 'X-group' on the enzyme participates,[3c] and a carbonium ion mechanism where loss of phosphate precedes C-H bond breaking.[6] Recently, Bartlett proposed a radical mechanism where abstraction of a hydrogen atom from C-6 first occurs to give an allyl radical **2**. Heterolytic cleavage of the phosphate group gives the radical cation **3** which upon single electron transfer affords chorismate **4**.[7]

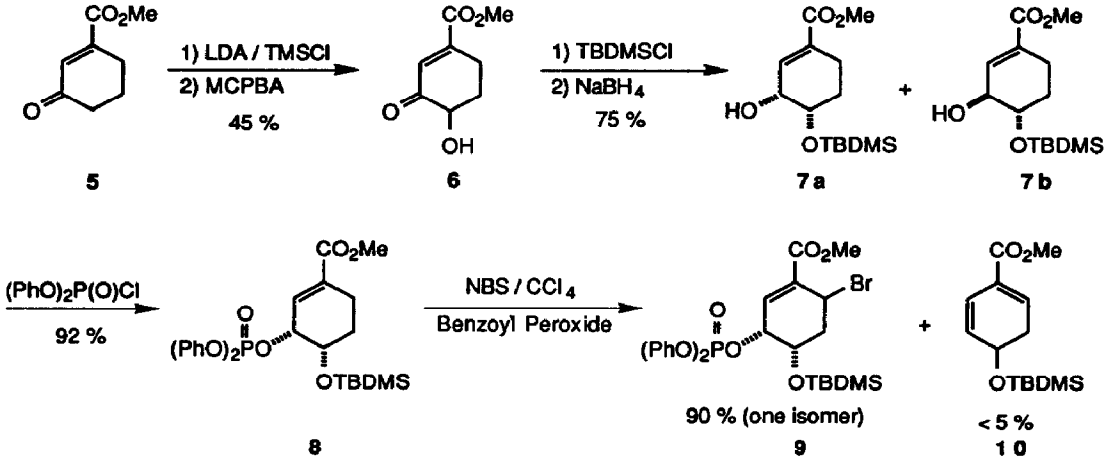


EPSP (**1**) **2** **3** Chorismate (**4**)

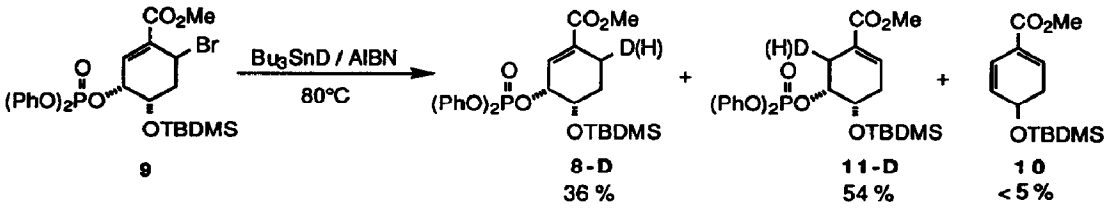
A radical mechanism would provide an explanation for the known requirements of a flavin cofactor and initial reduction of the enzyme. When experiments were performed with the substrate analog 6-fluoro-EPSP, a competitive inhibitor of *Neurospora crassa* chorismate synthase,[8] the $FMNH_2$ cofactor of *Escherichia coli* chorismate synthase was quantitatively oxidized to yield a stable flavin semiquinone radical[9] which was not detected with the natural substrate EPSP.[10] The authors proposed that a free radical could be a transient intermediate in the enzyme reaction with EPSP which does not build up when removal of a hydrogen atom from C-6 is possible. Therefore, we have carried out experiments with model systems to determine if a radical 1,4-elimination is possible in substituted cyclohexene systems. These experiments were designed to provide experimental evidence to either support or possibly exclude a radical mechanism of action for chorismate synthase.

The model system was synthesized as follows:[11] The enone **5** [12] was converted to the α-hydroxy ketone **6** in two steps by formation of the silyl enol ether followed by oxidation with MCPBA. Protection of the alcohol **6**

followed by reduction with $NaBH_4$ gave a 4:1 mixture of the allylic alcohols **7a** and **7b** which were separated by silica gel chromatography.[13] The phosphate ester **8** was prepared by treatment of the alcohol **7a** with diphenyl chlorophosphate and N-methylimidazole in dioxane. Allylic bromination of the phosphate ester with N-bromosuccinimide afforded the desired radical precursor **9** along with <5% of the diene **10**. The diene was presumably formed by initial hydrogen atom abstraction followed by elimination of the phosphate group. This reaction provided the first evidence that a 1,4-radical elimination is possible in this system.

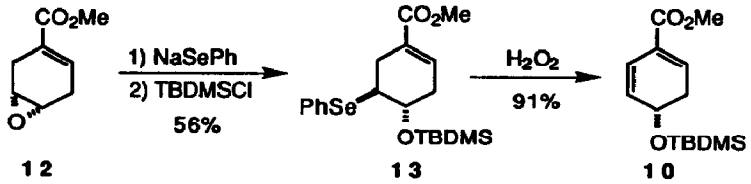


The allyl bromide **9** was then treated with a solution of Bu_3SnD/AIBN in refluxing benzene to determine if an allyl radical was formed upon abstraction of the bromine atom. The Bu_3SnD was added over 30 minutes by a syringe pump to a solution of **9** in benzene ($5 \cdot 10^{-2}$ M in **9** and $1 \cdot 10^{-1}$ in Bu_3SnD). The resulting phosphate esters **8-D** and **11-D** were obtained in 36 and 54% yield, respectively. A small quantity of the diene **10** was also formed. When the initiator AIBN was not present in the reaction mixture no reaction was observed. This result clearly indicates that an allyl radical is generated during the reaction of **9**. The reaction was next repeated under the same conditions using Bu_3SnH/AIBN. Again cyclohexenes (**8** and **11**) were formed as major products along with small amounts of cyclohexadiene **10**.

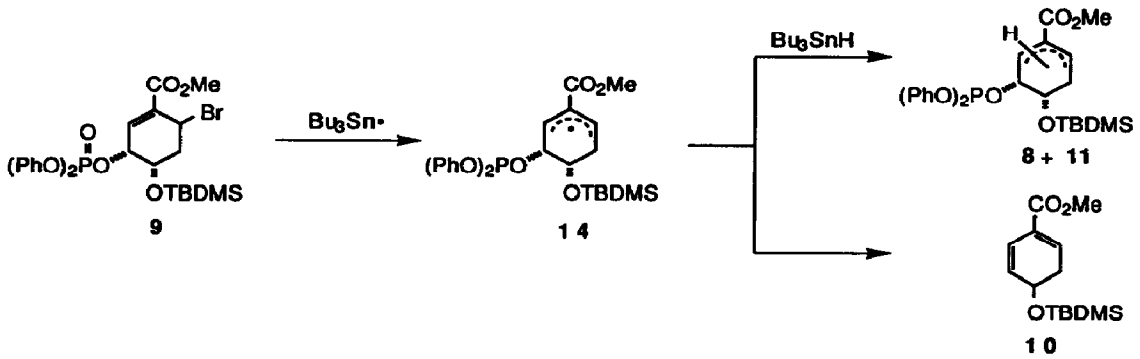


Reagents (M)		Addition Time (h)	Products (%)		
9	Bu_3SnH		**8**	**11**	**10**
$5 \cdot 10^{-2}$	$1 \cdot 10^{-1}$	0.5	34	53	<5
$1 \cdot 10^{-3}$	$1 \cdot 10^{-3}$	12	8	12	40

Apparently, the reaction of allyl radical with tin hydride (**14**→**8** + **11**)was occurring at a much faster rate than that of the corresponding elimination (**14**→**10**). Therefore, an experiment was performed using a low concentration of tin hydride. The Bu_3Sn/AIBN solution was slowly added to a solution of the bromide **9** in refluxing benzene (10^{-3} M in **9** and in Bu_3SnH) over 12 hours. A 40 % yield of the diene **10** along with 20% unreacted starting material and 20% of the reduced phosphate esters **8** and **11** were obtained. The structure of diene **10** was proved *via* an independent synthesis: The epoxide **12**[14] was ring opened with phenyl selenide anion (Ph_2Se_2, $NaBH_4$, MeOH)[15] to give a 2:1 mixture of hydroxy selenides which were separated by chromatography on silica gel. The alcohol was protected with TBDMSCl/imidazole and afforded the desired selenide **13**. Elimination of the selenide was accomplished by treatment with hydrogen peroxide to give the diene **10**. The resulting diene was shown to be identical to the diene formed by 1,4-elimination from the allyl bromide **9** by spectroscopic methods and GC-coinjection.



In order to determine the rate of the elimination step **14**→**10** a pseudo-first order kinetic study was performed using at least a fivefold excess of Bu_3SnH. The hydrogen abstraction **14**→**8**+**11** turned out to be $5 \cdot 10^3$ times faster (80°C, benzene) than the elimination step **14**→**10**. With a rate constant of about 10^5-10^6 $M^{-1} \cdot sec^{-1}$ for the hydrogen abstraction by related radicals[16] the elimination step **14**→**10** turns out to be about 10^2 $M^{-1} \cdot sec^{-1}$ under these conditions.



Conclusion: Allylphosphates can undergo 1,4 eliminations *via* allyl radicals. Product and rate studies with model system **9** are in accord with the Bartlett mechanism of the chorismate synthase reaction.

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