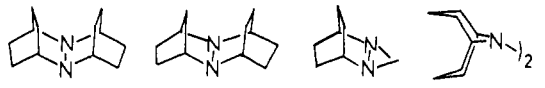
azo compound. If the azo compound were 10 pK_a units less basic than the product hydrazine, which seems entirely reasonable, this would provide 7 kcal/mol of exothermicity in the reaction with protonated azo compound compared to the reaction with unprotonated material at room temperature, which we propose is the reason for facile addition of the protonated azo compounds to cyclic dienes.

Hydrogenation of **2** and **4** to **6** and **7** using palladium on barium



6 7 8 9

carbonate in the presence of added potassium carbonate proceeds in excellent yield, although overreduction is a problem with more active catalysts. The four-step sequence reported here makes **6** available in 93% overall yield from **1**.

6 is the most easily oxidized hydrazine known; cyclic voltammetry measurements give $E°'(\mathbf{6},\mathbf{6}^{+}) = -0.53$ V,[6] making electron removal 0.60 V (13.8 kcal/mol) thermodynamically easier than oxidation of its monobicyclic analogue **8**.[7] We attribute its easy oxidation principally to strain relief upon removal of an antibonding electron. Hydrazines with 180° lone pair, lone pair dihedral angles such as **9** are known to have their nitrogens bent past tetrahedral geometry (average of the CNC and CNN angles less than 109.5°[8]), and we expect a 0° dihedral angle hydrazine like **6** to also electronically prefer very bent nitrogens. Such bending is resisted by methylene, methylene steric interaction in **6**, which makes neutral **6** quite strained. The radical cation $\mathbf{6}^{+}$ will have flattened nitrogens, relieving the steric interactions of the neutral form. As expected from the behavior of **9**, **6** shows a reversible second oxidation wave, $E°'(\mathbf{6}^{+},\mathbf{6}^{2+}) = 0.95$ V, making **6** 7.6 kcal/mol easier to oxidize to its dication than is **9**. $AgNO_3$ oxidation of **6** gives $\mathbf{6}^{+}NO_3^{-}$ in 97% yield as a faintly yellow solid, although other hydrazine radical cations we have worked with are distinctly yellow. $\mathbf{9}^{+}PF_6^{-}$ has a UV spectrum (CH_3CN) λ_m 340 (ϵ 4000), sh 260 nm (ϵ 1300),[9] while $\mathbf{6}^{+}PF_6^{-}$ absorbs at significantly shorter wavelength: (CH_3CN) λ_m 266 (ϵ 1700), 244 (ϵ 1700), 218 nm (ϵ 1800).[10] Two moles of $NOPF_6$ mixed with **6** in CH_3CN give $\mathbf{6}^{2+}(PF_6^{-})_2$, isolated as the CH_3CN solvate after precipitation by vapor diffusion of ether in 81% yield.[11] $\mathbf{6}^{2+}$ has ^1H NMR (CD_3CN) δ 6.23 (m, bridgehead), 2.73, and 2.11 (2m, CH_2) and ^{13}C NMR (CD_3CN) δ 81.4 and 30.3. Interestingly, the dication has longer wavelength absorption than the monocation radical: (CH_3CN) λ_m 317 (ϵ 2600), 227 nm (ϵ 8400); the same λ_m values are observed for $\mathbf{6}^{2+}(BF_4^{-})_2$. **6** is the first hydrazine for which three oxidation states are isolable, and structural data for these compounds will be reported when available. $\mathbf{6}^{2+}$ is remarkably kinetically stable and does not react rapidly with water. Its reactions with nucleophiles should prove interesting.

Acknowledgment. We thank the National Science Foundation for support of this work under Grant CHE-8026111.

Supplementary Material Available: Preparation of $\mathbf{2H}^{+}$, **2**, **6**, $\mathbf{6}^{+}NO_3^{-}$, and $\mathbf{6}^{2+}(PF_6^{-})_2$ (3 pages). Ordering information is given on any current masthead page.

(6) Cyclic voltammetry conditions: 0.1 M tetra-*n*-butylammonium perchlorate in acetonitrile, 23 ± 1 °C, 200 mV/s scan rate, Pt or Au working electrode, reported vs. a saturated calomel reference electrode.

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(8) (a) Nelsen, S. F.; Hollinsed, W. C.; Calabrese, J. C. *J. Am. Chem. Soc.* **1977**, *99*, 4461. (b) Nelsen, S. F.; Hollinsed, W. C.; Kessel, C. R.; Calabrese, J. C. *Ibid.* **1978**, *100*, 7876.

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(10) Interestingly, the UV of $\mathbf{6}^{+}$ is rather anion sensitive. The BF_4^{-} salt has λ_m 264 (ϵ 1600), 243 nm (ϵ 1500), and NO_3^{-} salt λ_m 267 (ϵ 1600), 203 nm (ϵ 9600), both in acetonitrile.

(11) NMR measurements make it likely that there are two CH_3N molecules per $\mathbf{6}^{2+}(PF_6^{-})_2$. The crystals lose solvent rapidly upon removal from CH_3CN, and our analysis corresponded to $\mathbf{6}^{2+}(PF_6^{-})_2 \cdot 1.8CH_3CN$. We have not obtained crystals with well-developed faces without CH_3CN present.

Steric Course of the 5-Enolpyruvylshikimate-3-phosphate Synthetase and Anthranilate Synthetase Reactions

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The reactions of the shikimate pathway of aromatic biosynthesis[1,2] pose a number of mechanistic and stereochemical problems. Three unanswered stereochemical questions center around the formation and further conversions of the key intermediate chorismate (**3**) and its immediate precursor, 5-enolpyruvylshikimate-3-phosphate (ESP) (**2**). These deal with the steric course of the conversion of phosphoenolpyruvate (**1**) into the enolpyruvyl side chain of **2** and with the direction of attack on the side-chain methylene group of **3** in the chorismate mutase and anthranilate synthetase reactions. Elucidation of these questions requires the generation of **2** and **3** labeled asymmetrically in the side-chain methylene group and is complicated by the fact that ESP synthetase operates by an addition/elimination mechanism.[3,4] We now report a solution to this problem.

An addition/elimination mechanism as shown in Scheme I will place a single, stereospecific tritium label from phosphoenolpyruvate evenly into the *E* and *Z* positions of the side chain of **2**. However, if every tritiated substrate molecule also carries deuterium in the other methylene position, the addition reaction will generate a chiral methyl group, which in the elimination step will produce two tritiated species, e.g., **2a** and **2b**, one containing deuterium and tritium and the other tritium and a normal hydrogen. Conversion of the methylene group of **2a** + **2b** into a methyl group by stereospecific introduction of ^1H will generate a "racemic" $C^1H_2{}^3H$ group from **2b** and a chiral methyl group from **2a**. The configuration of the latter will reveal the configuration of **2a**.[5]

To implement this approach (Scheme II) we synthesized (1*R*,2*R*)-[1-$^2H_1$,$^3H_1$]glycerol by equilibration of (2*R*)-2,3-isopropylidene-[1-$^2H_2$]glycerol[7] with alcohol dehydrogenase and [1-^3H]ethanol followed by acid hydrolysis.[9] The product was fed, together with excess unlabeled shikimate, to cultures of *Klebsiella pneumoniae* mutant 62-1 accumulating chorismic acid, using a modification of the conditions of Gibson.[10,11] The endogenously formed **1** will have *E* configuration;[12] it should produce **3** in which the molecules carrying ^2H and ^3H in the side chain have *Z* configuration if addition and elimination proceed with the

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(1) Haslam, E. "The Shikimate Pathway"; Wiley: New York, 1974.

(2) Weiss, U.; Edwards, J. M. "The Biosynthesis of Aromatic Compounds"; Wiley: New York, 1980.

(3) Levin, J. G.; Sprinson, D. B. *J. Biol. Chem.* **1964**, *239*, 1142.

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(5) If, as has since been demonstrated by Knowles and co-workers,[6] the ESP synthetase reaction involves a significant deuterium isotope effect, species **2a** will be formed in excess over **2b**, potentially allowing other strategies of analysis. Our approach, however, was designed to provide answers regardless of whether or not the elimination step proceeds with an isotope effect.

(6) Grimshaw, C. E.; Sogo, S. G.; Knowles, J. R. *J. Biol. Chem.* **1982**, *257*, 596.

(7) Obtained by $LiAlD_4$ reduction of the ethyl ester prepared from diisopropylidene-D-mannitol.[8]

(8) Baer, E.; Fischer, H. O. L. *J. Biol. Chem.* **1939**, *128*, 463.

(9) The presence of a large amount of unreacted dideuterated species is immaterial for the subsequent analysis.

(10) Gibson, F. *Biochem. Prep.* **1968**, *12*, 94.

(11) Earlier work[4] had suggested the possibility that **2** might bind to ESP synthetase and undergo reversible protonation/deprotonation. The in vivo approach was chosen to minimize this problem by tightly coupling the formation of **2** to its further conversion into **3**.

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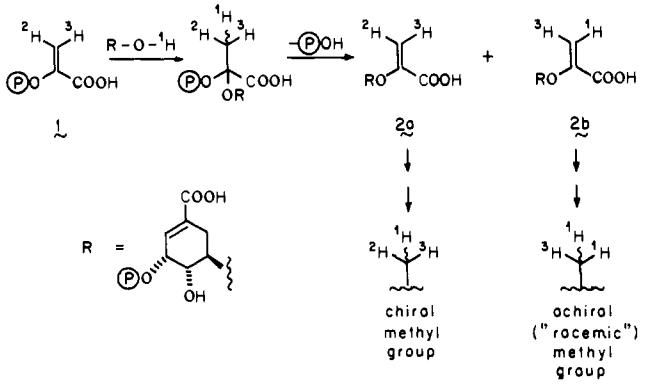
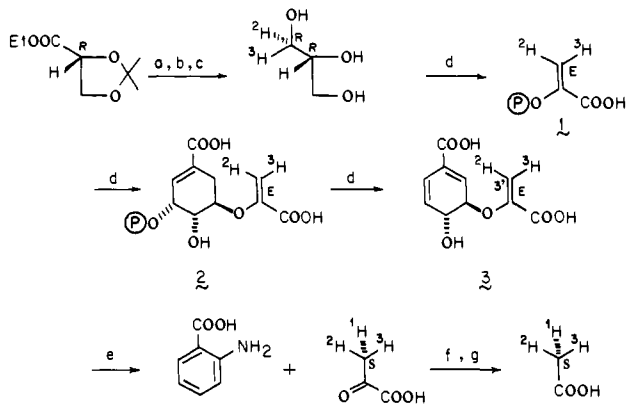
Scheme I



Scheme II[a]



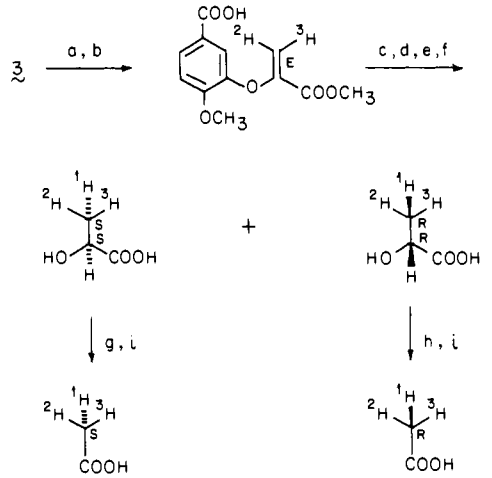
[a] (a) $LiAlD_4$; (b) liver alcohol dehydrogenase, [1-^{3}H]ethanol; (c) H^+; (d) *Klebsiella pneumoniae* 62-1; (e) anthranilate synthetase; (f) lactate dehydrogenase, NADH; (g) $Cr_2O_7^{2-}$, H^+.

same stereochemistry, e.g., both anti, or *E* configuration if the two steps proceed with opposite stereochemistry, i.e., one syn and one anti. To determine if it was indeed chirally labeled, aliquots of the product **3** were subjected to the action of anthranilate synthetase[13] in the presence of excess lactate dehydrogenase and NADH. The resulting lactate was oxidized to acetate,[14] which was analyzed for the configuration of the methyl group by the method of Cornforth et al.[15] and Arigoni and co-workers.[16] Observed *F* values[17] of 44.0 and 45.1 indicated the presence of 19% enantiomeric excess (ee) of *S* methyl groups.

The double-bond configuration of the deuterated, tritiated **3** was then determined by the reaction sequence shown in Scheme III. Aromatization,[18] reduction with Wilkinson's catalyst,[19] and Birch reduction[20] followed by hydrolysis gave a racemic mixture of lactate (10% yield) in which by virtue of the cis addition of hydrogen the configurations at C-2 and C-3 are correlated with each other. Incubation of an aliquot of the lactate with L-lactate dehydrogenase/NAD^+, followed by H_2O_2 oxidation of the resulting pyruvate[21] gave acetate, which in two separate experiments showed *F* values of 45.5 and 44.5 (16% and 19% ee *S*). Conversely, aliquots of the lactate upon oxidation with D-lactate dehydrogenase/NAD^+ and H_2O_2 gave acetate of $F = 55.1$ and 56.4 (18% and 22% ee *R*).

The results establish predominant *E* configuration for the side-chain double bond of the deuterated, tritiated **3** generated

Scheme III[a]



[a] (a) Pyridinium dichromate; DMF; (b) CH_2N_2; (c) tris(triphenylphosphine)rhodium chloride, H_2, benzene; (d) NaOH, CH_3OH; (e) Na, liquid NH_3; (f) dilute HCl, reflux; (g) L-lactate dehydrogenase, NAD^+; (h) D-lactate dehydrogenase, NAD^+; (i) H_2O_2.

from (*E*)-[3-$^2H_1$,$^3H_1$]-**1**, indicating that the addition and elimination steps in the ESP synthetase reaction proceed with opposite stereochemistry. This finding is consistent with a reaction path requiring a minimum of motion during the catalytic process. With the caveat that only one enantiomeric data set is available, the results also suggest that anthranilate synthetase catalyzed protonation on the *re* face of the enolpyruvyl side chain of **3**. This contrasts with the steric course of the reactions studied which involve attack at C-3 of phosphoenolpyruvate.[22]

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Copper-Catalyzed Acylation and Conjugate Addition of Zinc Homoenolate. Synthesis of 4- and 6-Oxo Esters

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Formation of carbon–carbon bonds by way of homoenolate anion has been a long-standing desire for organic chemists. A large portion of such an endeavor, some with the anion itself and usually with its equivalents, has been concentrated on 1,2-addition onto carbonyl compounds.[1] Feasibility and potentiality of conjugate addition, however, have not yet been demonstrated.[2] We are pleased to record here the first successful realization of such a reaction made possible by a copper-catalyzed reaction of zinc homoenolate **2** (eq 1). An expeditious synthetic route to γ-keto esters by C-acylation of the copper species is also reported.

The method for the conjugate addition is very simple yet highly efficient, consisting of in situ preparation of a mixture of the zinc

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(2) (a) With "equivalents"; Lesur, B.; Toye, J.; Chantrenne, M.; Ghosez, L. *Tetrahedron Lett.* **1979**, 2835. De Lombaert, S.; Lesur, B.; Ghosez, L. *Ibid.* **1982**, *23*, 4251. Evans, D. A.; Billargeon, D. J.; Nelson, T. V. *J. Am. Chem. Soc.* **1978**, *100*, 2242. (b) Homoenolate "radicals" add to enones of limited structural types: Giese, B.; Horner, H. *Tetrahedron Lett.* **1983**, *24*, 3221.

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