Divergence between the Enzyme-Catalyzed and Noncatalyzed Synthesis of 3-Dehydroquinate

Paul A. Bartlett,* Kevin L. McLaren, and Matthew A. Marx

Department of Chemistry, University of California, Berkeley, California 94720

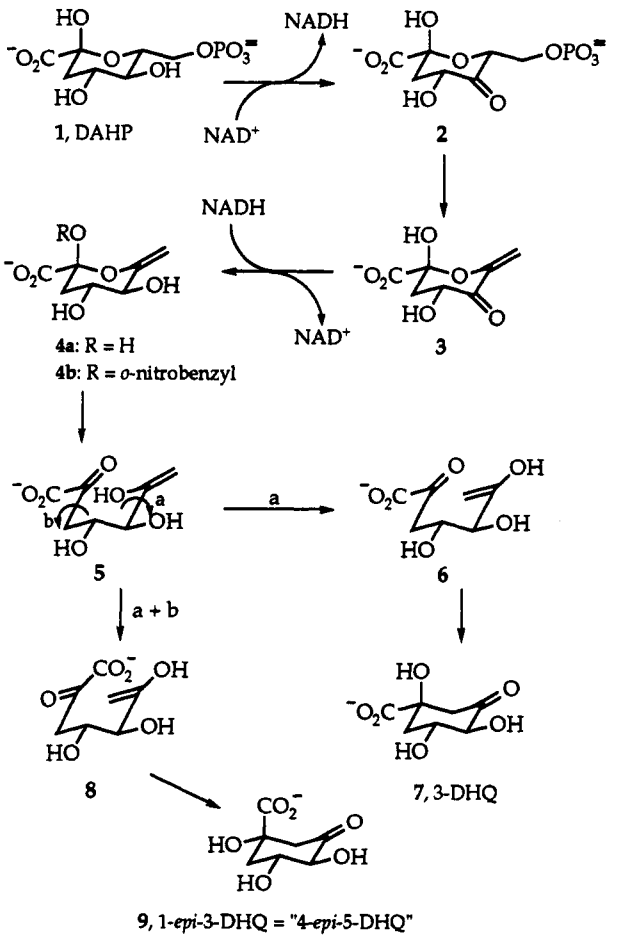
Received October 15, 1993•

Synthesis of 1-*epi*-dehydroquinate, **9**, provided an authentic sample of this material and allowed its identification as a minor product in the noncatalyzed rearrangement of enolpyranose **4a** to 3-dehydroquinate, **7** (3-DHQ). None of this isomer was detected in the product of the transformation of DAHP, **1**, to 3-DHQ catalyzed by dehydroquinate synthase. This result indicates that the enolpyranose **4a** is not released from the enzyme active site prior to rearrangement to 3-DHQ, a possibility suggested previously (Bartlett, P. A.; Satake, K. *J. Am. Chem. Soc.* **1988**, *110*, 1628–1630). Enolpyranose **4a** was generated in the presence of DHQ synthase; however, the formation of **9** was not diminished, indicating that spontaneous rearrangement is faster than uptake by the enzyme under these conditions. The question remains whether the enzyme takes an active role in catalyzing the rearrangement of **4a** to 3-DHQ or simply provides a conformational template to prevent formation of the side product **9**.

3-Dehydroquinate (3-DHQ) synthase (E.C. 4.6.1.3) catalyzes the transformation of 2-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP, **1**) into 3-DHQ, **7**, by a mechanism that involves at least five distinct steps (Scheme 1).[1] The awe with which this multitalented enzyme was regarded initially has been tempered by suggestions that the steps involving elimination of phosphate from **2**,[2] ring opening of the enolpyranose **4a**, and final aldol condensation[3] may not require enzymatic intervention, i.e., that they occur spontaneously. It has been shown, for example, that enolpyranose **4a**, generated by photochemical deprotection of nitrobenzyl acetal **4b**, rearranges rapidly in aqueous solution to give 3-DHQ.[3] This observation raised the possibility that **4a** may be the actual product of the enzymatic reaction. According to this minimalist view, DHQ synthase may be responsible only for the oxidation and reduction steps involving the C5 position of DAHP.[4] To probe this question further, we undertook a more detailed study of the noncatalyzed rearrangement of enolpyranose **4a** to see if it differed in any detail from the biosynthetic process. The finding of any such difference would indicate that the enzyme plays a role in the last steps of the transformation.

The stereochemical course of the phosphate elimination[5] and the fate of the hydrogens at C7 (DAHP numbering)[1b] indicate that the cyclization reaction proceeds via a chairlike transition state (Scheme 1), requiring a 180° rotation about the C5–C6 bond after opening of the pyranose ring (**5** → **6**). Studies with a deuterium-labeled form of precursor **4b** demonstrated that the noncatalyzed rearrangement also proceeds via this transition state,[3] but they were not accurate enough to rule out the possibility that small amounts of product are formed by other stereochemical pathways, either via the boatlike transition state[6] or as a result of rotation around the C2–C3 bond (**5** → **8**). In this report, we describe our search for the product of the latter pathway, 1-*epi*-3-DHQ, **9**, and demonstrate

Scheme 1



• Abstract published in *Advance ACS Abstracts*, March 15, 1994.

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(2) (a) Widlanski, T.; Bender, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1989**, *111*, 2299–2300. (b) Bender, S. L.; Widlanski, T.; Knowles, J. R. *Biochemistry* **1989**, *28*, 7560–7572.

(3) Bartlett, P. A.; Satake, K. *J. Am. Chem. Soc.* **1988**, *110*, 1628–1630.

(4) Knowles, J. R. *Aldrichim. Acta* **1989**, *22*, 59–66.

(5) (a) Widlanski, T.; Bender, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 1873–1875. (b) Widlanski, T.; Bender, S. L.; Knowles, J. R. *Biochemistry* **1989**, *28*, 7572–7582.

(6) The stereochemical purity and level of deuterium label incorporation in the labeled form of **4b** limited the accuracy with which the chair/boat distinction could be made.[3]

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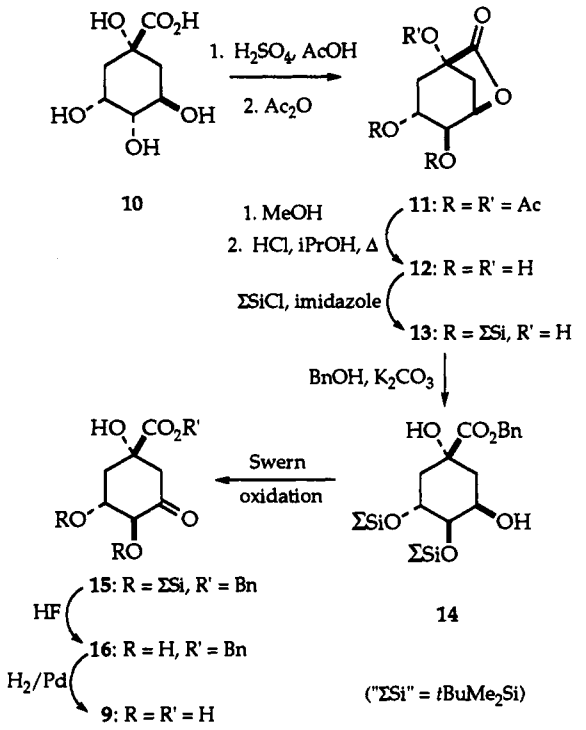
Scheme 2



that it is formed in different amounts in both the enzyme-mediated and the spontaneous rearrangements of enolpyranose **4a**.

1-*epi*-3-DHQ has not been reported previously; to obtain authentic material for identification, we developed a synthesis starting from quinic acid (Scheme 2). The foundation of this synthesis was the recognition that 1-*epi*-3-dehydroquinic acid is equivalent to "4-*epi*-5-dehydroquinic acid" (Scheme 1). We anticipated that epimerization at C4 would be more straightforward than epimerization at C1 and devised our synthesis accordingly. Solvolysis of quinic acid in H_2SO_4/acetic acid results in a mixture of products arising from epimerization, lactonization, and random acetylation,[7] including the triacetate **11**, which crystallizes conveniently from the reaction mixture in 12% yield. Methanolysis and relactonization provide the triol **12** in 36% yield. Protection with *tert*-butyldimethylsilyl chloride gives a mixture of isomers from which the diether **13** can be obtained in 36% yield, thereby differentiating the secondary hydroxyl groups. Lactone opening with benzyl alcohol and Swern oxidation afford the protected form of 1-*epi*-3-DHQ, **15**, in 43% yield. Desilylation with either aqueous HF or tetrabutylammonium fluoride in THF leads to β-elimination in competition with deprotection; however, HF in acetonitrile gives the ketotriol **16** cleanly. Finally, hydrogenolysis of the benzyl ester affords 1-*epi*-3-DHQ, **9**, as a white solid.

Although we were not able to distinguish 1-*epi*-3-DHQ from 3-DHQ itself by HPLC, these compounds are strikingly different by ^{1}H NMR: while the methylene hydrogens at C2 in 3-DHQ resonate at δ 2.45 and 3.05 ppm (J = 14 Hz) in D_2O at pH 8.0 (Figure 1c), those in 1-*epi*-3-DHQ are magnetically coincident, appearing as a single broad peak at δ 2.73 ppm (Figure 1a). Because of this fortuitous difference, even a few percent of 1-*epi*-3-DHQ can be detected in the presence of 3-DHQ.

Photolytic deprotection of the precursor **4b** in phosphate buffers at pH's from 5.0 to 9.0 leads to 3-DHQ as the major product, as reported previously.[3] Knowledge of where 1-*epi*-3-DHQ appears in the ^{1}H NMR spectrum allows 2.5–4.0% of this isomer to be identified in these photolysis mixtures (Figure 1b). Spiking an NMR sample of material generated in this fashion with authentic 1-*epi*-3-DHQ confirmed the assignment of the minor peaks at δ 2.08, 2.73, and 4.16 ppm as due to this isomer. The amount of the 1-*epi*-material formed does not appear to be dependent on pH, nor is it formed from epimerization of 3-DHQ itself under the photolysis conditions. Finally, formation of the epimeric product cannot be attributed to contamination of **4b** by its anomer; although we did not have an authentic sample, no more than 1% of this isomer could be present according to the ^{1}H NMR spectrum of the precursor.[8]

To determine whether 1-*epi*-3-DHQ is formed in the enzymatic reaction, the crude product from conversion of 3.0 mg of DAHP to 3-DHQ with 3 mg of DHQ synthase was filtered (MW cutoff 10 000 D) and the filtrate was examined by ^{1}H NMR. It is apparent that the epimeric material is not formed in the enzymatic process to the same extent it is from the spontaneous rearrangement of enolpyranose **4a**, if it is formed at all (Figure 1c).[9] This difference points strongly to an involvement of the enzyme through the last step in the synthesis of 3-DHQ.

The photochemical deprotection of **4b** was carried out over a 3-h period in the presence of up to 750 μM DHQ synthase, in an attempt to see uptake of the enolpyranose by the enzyme and a possible diminution in the amount of the epimeric product formed. No change in the amount of 1-*epi* material was observed, however (data not shown). These experiments were carried out in the presence of a large amount of dehydroquinase to catalyze the conversion of 3-DHQ to dehydroshikimate and prevent the possible inhibition of DHQ synthase by buildup of product, and it was shown that 1-*epi*-3-DHQ is not a substrate for dehydroquinase nor an inhibitor of either enzyme. Furthermore, it was demonstrated that photolysis of an equivalent amount of *o*-nitrobenzyl alcohol does not lead to inhibition of the enzymes.

The fact that the presence of enzyme does not reduce the amount of 1-*epi*-3-DHQ generated by photodeprotection of **4b** indicates that spontaneous rearrangement of the enolpyranose is faster than its rate of association with DHQ synthase. It has been pointed out that reactive intermediates are often prevented from dissociating from the enzyme by kinetic, as opposed to thermodynamic, barriers;[10] such a barrier, which must slow the association rate as well, may be involved in this case, or the rearrangement may simply be too fast.

Discovery of 1-*epi*-3-DHQ in the solution rearrangement of the enolpyranose **4a**, but not in the enzyme-mediated process, indicates that this intermediate is not released from the enzyme prior to rearrangement. It remains an open question as to whether the enzyme is actively involved in catalyzing this rearrangement or simply serves as a template to ensure that it pursues the proper stereochemical course. A number of considerations point to the latter interpretation. For example, the speed with which **4a** rearranges to 3-DHQ indicates that catalysis is not

(7) (a) Gorin, P. A. J. *Can. J. Chem.* **1963**, *41*, 2417–2423. (b) Corse, J.; Lundin, R. E. *J. Org. Chem.* **1970**, *35*, 1904–1909.

(8) We thank one of our reviewers for bringing this possibility to our attention.

(9) Similar results have been observed in unpublished work by Knowles and Cheng (J. R. Knowles, personal communication).

(10) Cleland, W. W. *Biochemistry* **1990**, *29*, 3194–3197.

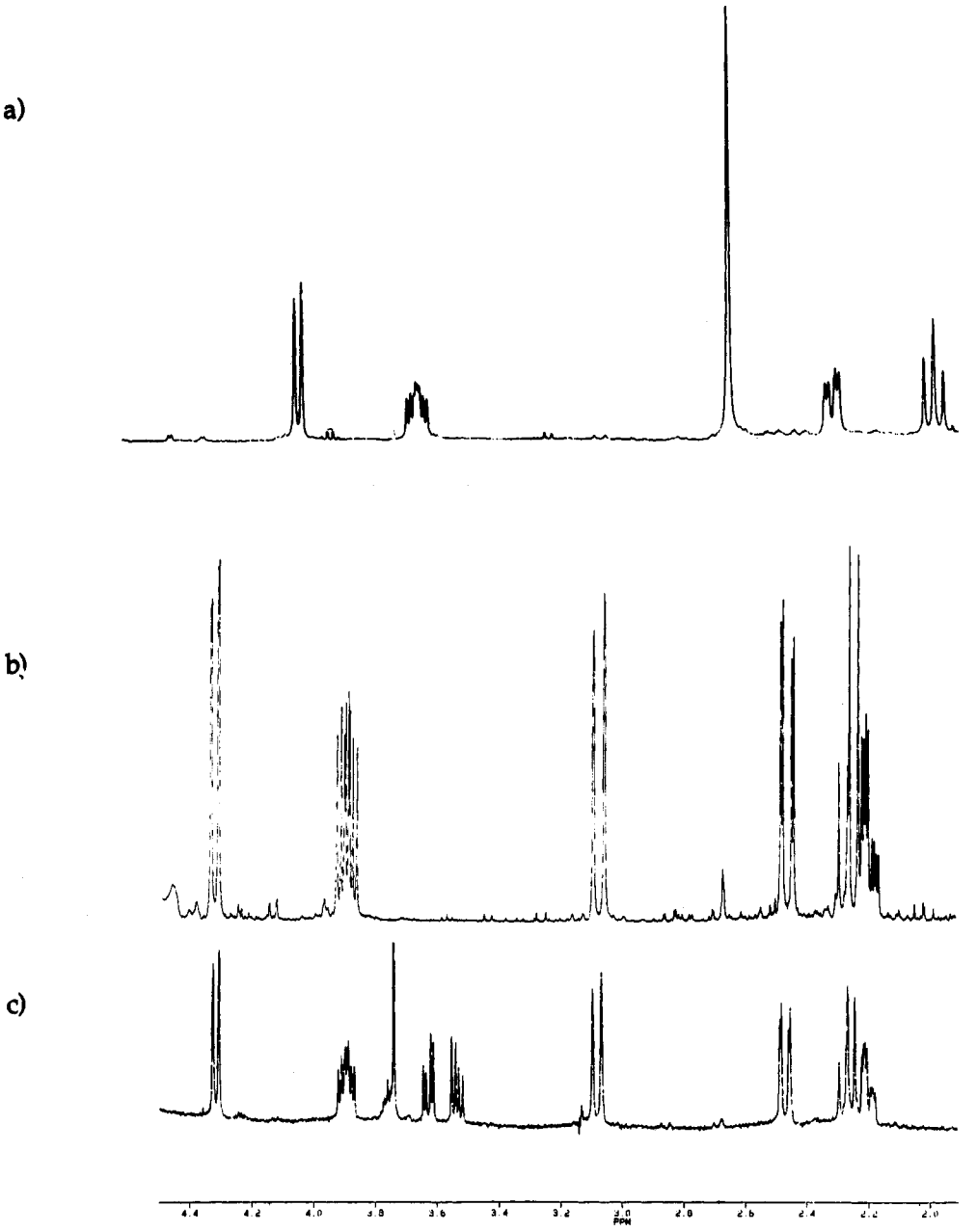


Figure 1. ^{1}H NMR spectra of (a) 1-*epi*-3-DHQ, (b) product from photolysis of the enolpyranose precursor **4b** at pH 9.0, and (c) authentic 3-DHQ produced by enzymatic conversion of DAHP. The extraneous peaks in spectrum c from δ 3.5–3.8 ppm correspond to the glycerol used to stabilize the enzyme and dioxane as an internal standard.

required for this step. Moreover, it is likely that interaction of the carboxylate moiety with the enzyme active site is an important factor in substrate binding; this interaction may well prevent rotation of the α-keto acid moiety in **5**/**6** and thereby control the configuration of the 3-DHQ product. Rather than marvel at the catalytic versatility of DHQ synthase, perhaps we should admire instead its cunning in exploiting the spontaneous reactions of the intermediates it generates.

Experimental Section

(1*S*,3*R*,4*S*,5*R*)-1,3,4-Triacetoxy-6-oxabicyclo[3.2.1]octan-7-one (11).[7] A solution of 20.0 g (104 mmol) of quinic acid, **10**, and 14 mL (26 g, 249 mmol) of concd H_2SO_4 in 1 L of AcOH was heated at reflux for 100 h. The mixture was cooled to 0 °C, 1 L of Ac_2O was added slowly, and the solution was stirred at 21 °C for 20 h. The solution was poured onto 3 L of ice, and the mixture was allowed to warm to 21 °C with stirring for 1 h. The aqueous solution was extracted six times with 250 mL of CH_2Cl_2, and the combined organic layer was dried (Na_2SO_4), filtered, and concentrated to an oil which was evaporated from heptane to yield 4.3 g of a gray solid. Crystallization of this material from ethyl acetate/hexanes gave 3.7 g (12%) of triacetate **11** as tan prisms: mp 171–177 °C [lit.[7] mp 182–183 °C]; ^{1}H NMR (400 MHz) δ 2.03 (s, 3), 2.09 (s, 3), 2.10 (m, 1), 2.11 (s, 3), 2.22 (d, 1, J = 11.7 Hz), 2.54 (ddd, 1, J = 3.5, 7.7, 12.2 Hz), 3.23 (ddd, 1, J = 3.5, 7.1, 11.6 Hz), 4.83 (dd, 1, J = 1.2, 7.0 Hz), 4.94 (dd, 1, J = 1.3, 8.5 Hz), 5.12 (ddd, 1, J = 7.7, 8.3, 10.6 Hz).

(1*R*,3*R*,4*R*,5*R*)-3,4-Bis[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-6-oxabicyclo[3.2.1]octan-7-one (13). A solution of 1.06 g (3.53 mmol) of lactone triacetate **11** and 49 mg (0.35 mmol) of K_2CO_3 in 35 mL of MeOH was stirred at 21 °C for 3 h. A 12 N solution of HCl (90 μL, 1.1 mmol) was added dropwise, the solvent was removed under reduced pressure, and the residue was evaporated twice from 30 mL of benzene. The resulting glass was dissolved in 20 mL of *i*-PrOH and heated at 80 °C for 1 h. After the mixture cooled, a gray precipitate was collected by filtration and lyophilized from 20 mL of H_2O to give 210 mg

(34%) of lactone triol **12** as a white powder: mp 190–197 °C; ^{1}H NMR (250 MHz, DMSO) δ 1.60 (dd, 1, J = 10.5, 12.2 Hz), 1.94 (m, 1), 1.97 (d, 1, J = 11.3 Hz), 2.27 (ddd, 1, J = 3.5, 6.7, 11.3 Hz), 3.26–3.40 (m, 2), 4.45 (d, 1, J = 6.6 Hz), 5.10 (br s, 1), 5.25 (br s, 1), 5.92 (br s, 1); ^{1}H NMR (250 MHz, H_2O) δ 1.77 (dd, 1, J = 10.4, 12.2 Hz), 2.09 (d, 1, J = 11.9 Hz), 2.11 (ddd, 1, J = 3.6, 6.9, 12.8 Hz), 2.44 (ddd, 1, J = 3.6, 6.9, 11.9 Hz), 3.48–3.61 (m, 2), 4.66 (d, 1, J = 6.9 Hz).

A solution of 180 mg (1.2 mmol) of *tert*-butyldimethylsilyl chloride, 350 mg (5.1 mmol) of imidazole, and 180 mg (1.03 mmol) of lactone **12** in 3.5 mL of DMF was stirred at 21 °C for 8 h, at which point an additional 170 mg (1.1 mmol) of *t*-BDMS chloride was added. After 12 h, the reaction mixture was partitioned between ethyl acetate and cold 0.3 N HCl. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to give 281 mg of a pale oil. Chromatography (4:1 hexanes/EtOAc) of this product gave 35 mg (7%) of the tris(silylated) product, 87 mg (29%) of the 4- and 5-monosilylated isomers, and 149 mg (36%) of the desired 4,5-bis(*t*-BDMS) lactone **13** as white needles: mp 141–145 °C; IR 3570, 2960, 2930, 2900, 2860, 1785, 1465, 1255, 1250, 1115, 1095, 950, 830 cm^{-1}; ^{1}H NMR (250 MHz) δ 0.036 (s, 3), 0.045 (s, 3), 0.085 (s, 6), 0.86 (s, 9), 0.90 (s, 9), 1.85 (dd, 1, J = 10.5, 12.5 Hz), 2.09 (d, 1, J = 11.6 Hz), 2.12 (ddd, 1, J = 3.5, 7.0, 12.5 Hz), 2.46 (ddd, 1, J = 3.6, 6.8, 11.5 Hz), 3.59 (dd, 1, J = 1.1, 7.3 Hz), 3.72 (ddd, 1, J = 7.1, 7.2, 10.4 Hz), 4.54 (dd, 1, J = 1.1, 7.3 Hz); HRMS FAB$^+$ (M + H)$^+$ calcd for $C_{19}H_{39}O_5Si_2$ 403.2336, found 403.2340.

Phenylmethyl [1*S*-(1α,3β,4β,5α)]-4,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1,3-dihydroxy-1-cyclohexanecarboxylate (14). A mixture of 345 mg (0.857 mmol) of the disilylated lactone **13** and 6 mg (0.04 mmol) of K_2CO_3 in 1.8 mL (1.9 g, 17 mmol) of benzyl alcohol was stirred at 21 °C for 16 h. Direct chromatography of this mixture (0:100–1:99 MeOH/CH_2Cl_2) gave 900 mg of crude ester contaminated with benzyl alcohol. The mixture was lyophilized from 30 mL of H_2O to yield 400 mg of a colorless glass. Chromatography (0.5:99.5 MeOH/CH_2Cl_2) gave 354 mg (81%) of pure benzyl ester **14** as a glass: IR 3490, 2960, 2940, 2900, 2870, 1745, 1260, 1225, 1220, 1115, 1100, 1075 cm^{-1}; ^{1}H NMR (250 MHz) δ 0.085 (s, 3), 0.105 (s, 6), 0.113 (s, 3), 0.88 (s, 9), 0.89 (s, 9), 1.6 (br s, 1), 1.76 (ddd, 1, J = 2.1, 2.1, 14.4 Hz), 1.96 (dd, 1, J = 11.5, 12.3 Hz), 2.10 (ddd, 1, J = 2.0, 4.3, 12.3 Hz), 2.25 (dd, 1, J = 2.6, 14.4 Hz), 3.84 (dd, 1, J = 2.9, 3.1 Hz), 4.08 (ddd, 1, J = 3.0, 3.1, 3.5 Hz), 4.15 (ddd, 1, J = 2.8, 4.7, 11.2 Hz), 4.42 (s, 1), 5.14 (d, 1, J = 12.5 Hz), 5.23 (d, 1, J = 12.5 Hz), 7.33 (m, 5). Anal. Calcd for $C_{26}H_{46}O_6Si_2$: C, 61.13; H, 9.08. Found: C, 60.94; H, 9.04.

Phenylmethyl [1*S*-(1α,3α,4β)]-3,4-Bis[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-5-oxo-1-cyclohexanecarboxylate (15). DMSO (0.30 mL, 0.33 g, 4.2 mmol) was added dropwise to a solution of 0.18 mL (0.26 g, 2.1 mmol) of oxalyl chloride in 7 mL of CH_2Cl_2 at −78 °C. After 15 min, a solution of 313 mg (0.613 mmol) of alcohol **14** in 7 mL of CH_2Cl_2 was added dropwise over a 15-min period. After 30 min at −78 °C, 1.4 mL (1.0 g, 10 mmol) of Et_3N was added, and the solution was stirred for 45 min. Saturated NH_4Cl was added at −78 °C, and the mixture was stirred for 15 min while being warmed to room temperature and then partitioned between ethyl acetate and cold 0.3 N HCl. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated to yield 316 mg of a clear glass. Chromatography (15:85 EtOAc/hexanes) of this material gave 165 mg (53%) of ketone **15** as a colorless glass: IR 3480 (br), 2960, 2940, 2900, 2870, 1735, 1260, 1255, 1245, 1095 cm^{-1}; ^{1}H NMR (250 MHz) δ 0.020 (s, 3), 0.059 (s, 3), 0.065 (s, 3), 0.080 (s, 3), 0.86 (s, 18), 2.01 (ddd, 1, J = 2.0, 3.6, 14.4 Hz), 2.59 (ddd, 1, J = 0.9, 1.9, 13.6 Hz), 2.64 (dd, 1, J = 2.6, 14.4 Hz), 3.30 (d, 1, J = 13.6 Hz), 3.81 (d, 1, J = 4.5 Hz), 4.18 (ddd, 1, J = 2.8, 4.2, 4.3 Hz), 4.44 (br s, 1), 5.16 (d, 1, J = 12.3 Hz), 5.26 (d, 1, J = 12.3 Hz), 7.34 (m, 5). Anal. Calcd for $C_{26}H_{44}O_6Si_2$: C, 61.38; H, 8.72. Found: C, 61.32; H, 8.81.

[1*S*-(1α,3α,4β)]-5-Oxo-1,3,4-trihydroxy-1-cyclohexanecarboxylic Acid (1-*epi*-3-Dehydroquinic Acid, 9). A solution of 116 mg (0.228 mmol) of the disilyl ketone **15** in 11 mL of CH_3CN was cooled to 0 °C, placed under an anhydrous HF atmosphere for 30 s, and then stirred for 5 min. Direct chromatography (CH_3CN, with silica gel prewashed with distilled pyridine) gave 68 mg (99+% yield) of benzyl 1-*epi*-3-dehydroquinate **16** as a colorless glass that crystallized on standing: mp 80–82 °C; ^{1}H NMR (250 MHz) δ 2.18 (dd, 1, J = 11.6, 13.9 Hz), 2.39 (ddd, 1, J = 2.4, 5.0, 14.0 Hz), 2.70 (br s, 1) 2.72 (dd, 1, J = 1.3, 14.8 Hz), 2.87 (dd, 1, J = 2.4, 14.8 Hz), 3.52 (br s, 1), 3.55 (br s, 1), 3.75 (m, 1), 4.05 (dd, 1, J = 1.6, 8.3 Hz), 5.14 (d, 1, J = 12.0 Hz), 5.20 (d, 1, J = 12.0 Hz), 7.36 (m, 5).

A suspension of 53.9 mg (0.192 mmol) of ester **16** and 19 mg of 10% palladium on carbon in 19 mL of EtOAc was stirred under an atmosphere of hydrogen for 2 h at 21 °C. The catalyst was removed by filtration through Celite, and the solution was evaporated to give 33.3 mg (91% yield) of 1-*epi*-3-DHQ **9** as a white, waxy solid: ^{1}H NMR (400 MHz, D_2O, referenced to dioxane as 3.74 ppm) δ 2.08 (dd, 1, J = 12.0, 13.1 Hz), 2.40 (dd, 1, J = 4.9, 13.3 Hz), 2.73 (s, 2), 3.87 (ddd, 1, J = 4.9, 9.3, 11.7 Hz), 4.16 (d, 1, J = 9.3 Hz); ^{13}C NMR (100 MHz, D_2O, referenced to sodium 3-(trimethylsilyl)-1-propanesulfonate) δ 41.84, 48.88, 69.86, 73.43, 80.31, 177.50, 202.15; HRMS FAB$^-$ (M − H)$^-$ calcd for $C_7H_9O_6$ 189.0399, found 189.0401.

Photolytic Generation of Enolpyranose 4a. In a typical experiment, a solution of 17 mg (52 μmol) of the nitrobenzyl acetal **4b** in 1.6 mL of phosphate buffer at 0 °C was irradiated for 2 h with an Ace-Hanovia 450-W Hg lamp. The amount of 1-*epi*-3-DHQ was determined by integration of the appropriate ^{1}H NMR peaks. Separate experiments were carried out at pH 5, 6, 7, 8, and 9.

Enzymatic Preparation of Dehydroquinate. Dehydroquinate synthase (DHQ synthase) was obtained as a solution of 12 mg/mL (18 units/mg), stabilized with glycerol. Standard conditions for the enzymatic reactions have been reported.[11]

Generation of Enolpyranose in the Presence of DHQ Synthase. In a typical experiment, a solution containing 5.0 mg of nitrobenzyl acetal **4b** in 1.0 mL of phosphate buffer, pH 8, was combined with a solution of DHQ synthase (2.2 mg, 43 units) prepared in 0.5 mL of phosphate buffer at pH 8 with 750 μmol of Co^{+2} and 1.5 mmol of NAD^+. The resulting solution was irradiated as described above at 0 °C. After 2 h, the solution was centrifuged through a Centricon filter (designed to retain species >10 000 MW), and the ratio of isomers was determined by ^{1}H NMR.

Acknowledgment. We thank Professor J. R. Knowles (Harvard University) for valuable discussions and for generous gifts of DHQ synthase and dehydroquinase, as well as George Lucier (UCB) for assistance with the HF line. This work was supported by the National Institutes of Health (Grant No. GM-28965) and a fellowship from the Department of Education (to M.A.M.).

(11) Mehdi, S.; Frost, J. W.; Knowles, J. R. *Methods Enzymol.* **1987**, *142*, 306–314. Bender, S. L.; Mehdi, S.; Knowles, J. R. *Biochemistry* **1989**, *28*, 7555–7560.