Enzymatic and Nonenzymatic Reactions of 1β-[(1-Carboxyethenyl)oxy]-4α-hydroxycyclohex-2-ene-1-carboxylate

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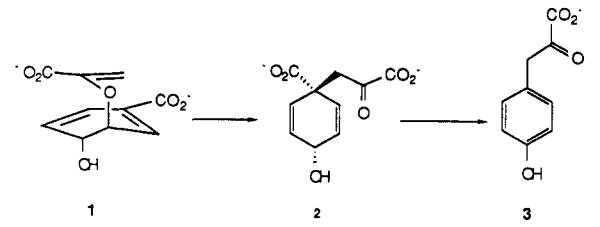
The synthesis of disodium 1β-[(1-carboxyethenyl)oxy]-4α-hydroxycyclohex-2-ene-1-carboxylate (**4**) is described. Compound **4** was synthesized in five steps from 1,4-diol **10**, which was prepared in three steps from the known diene **7**. Compound **4** undergoes a facile Claisen rearrangement ($T_{1/2}$ = 7 h, 30 °C, pD = 7.4) and unlike chorismate shows a slight pH dependence upon rate of rearrangement ($T_{1/2}$ = 1.0 h, 30 °C, pD = 1.5). This dependence is explained in terms of the sensitivity of C-1 of **4** to electronic substituent effects. Analogue **4** is not a substrate for chorismate mutase but is a reasonable substrate for prephenate dehydrogenase (V_{max} = 0.16 μmol min^{-1} mg^{-1}). Compounds **4** and **5** were found to be competitive inhibitors of chorismate mutase (K_i = 1.28 mM and 0.97 mM, respectively).

Chorismate (**1**) is the last common intermediate in the biosynthesis of aromatic amino acids via the shikimate pathway.[1] The first step in the synthesis of phenylalanine and tyrosine from **1** is the Claisen rearrangement to prephenate (**2**). The rearrangement proceeds readily without catalysis ($t_{1/2}$ = 15.7 h, 30 °C, pH 7.5) and is accelerated by a factor of 1.9 × 10^6 by the enzyme chorismate mutase.[2] Both the uncatalyzed[3] and the enzyme-catalyzed[4,5] rearrangements of **1** to **2** proceed through chair-like geometry in the transition state (Scheme I). In the biosynthesis of tyrosine, prephenate dehydrogenase, an NAD$^+$-requiring enzyme, catalyzes the conversion of **2** to (*p*-hydroxyphenyl)pyruvate (**3**).[1]

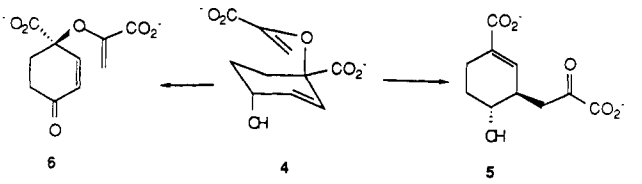
Despite numerous studies, the catalytic action of chorismate mutase is not understood. The possibility that chorismate mutase simply binds the diaxial conformer of chorismate in the chair-like conformation for rearrangement does not provide sufficient transition-state stabilization to account for the rate acceleration observed in the enzyme-catalyzed reaction.[6,7] The manner in which chorismate mutase could possibly effect the observed rate enhancement has been discussed recently by Knowles and co-workers.[7] Although acid catalysis of [3,3]-sigmatropic rearrangements has been observed,[8] enzymic catalysis by provision of an acidic center near the enolpyruvyl oxygen atom was not considered a reasonable mechanism since previous work by the Knowles group demonstrated that there is no detectable secondary tritium isotope effect at C-5.[9] The enzyme-catalyzed pathway suggested by the Knowles group, and consistent with available evidence, involves rate-limiting cleavage of the C-5–oxygen bond with assistance by addition of a nucleophilic center on the enzyme at C-5, to provide an intermediate that collapses to **2** by an S_n2' process.[7]

Chorismate-prephenate analogue **4** (Scheme II) was of interest to us since conceivably it could be a substrate for chorismate mutase and/or prephenate dehydrogenase.

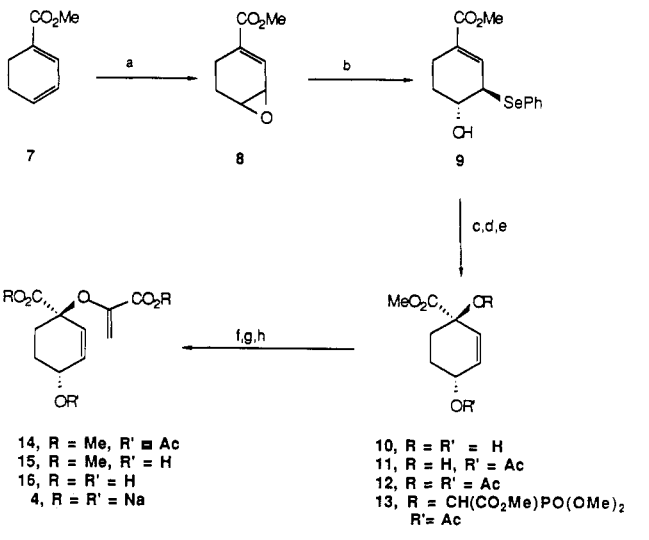
Scheme I



Scheme II



Scheme III[a]



14, R = Me, R' = Ac
15, R = Me, R' = H
16, R = R' = H
4, R = R' = Na

10, R = R' = H
11, R = H, R' = Ac
12, R = R' = Ac
13, R = $CH(CO_2Me)PO(OMe)_2$
R' = Ac

[a] Key: (a) *m*-CPBA/CH_2Cl_2; (b) $Ph_2Se_2/NaBH_4$, 0 °C; (c) 30% H_2O_2/acetone; (d) Ac_2O, Et_3N/CH_2Cl_2; (e) $MeO_2CC(N_2)PO(OMe)_2/Rh_2(O_2CC_7H_{15})_4$/benzene, 80 °C; (f) $LiN(SiMe_3)_2/H_2CO$, −65 °C; (g) NaOMe/MeOH, 0 °C; (h) NaOH, THF/H_2O.

On the basis of substituent effects,[10–12] it was expected that the rate of Claisen rearrangement of **4** would be sim-

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(3) Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 5306–5308.

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ilar to that of 1. That 4 might adopt the requisite chair-like geometry for binding to the mutase site (Scheme II) appeared reasonable, but catalysis of the rearrangement of 4 to 5 would not be expected if the mutase-catalyzed rearrangement of 1 involved enzymic protonation of the enolpyruvyl oxygen atom or if the Knowles suggestion of assistance of a nucleophilic site on the enzyme were involved.[13] If binding energy contributes substantially to the acceleration of the Claisen rearrangement of 1, efficient binding of 4 might be sufficient to catalyze the rearrangement. On the other hand, since both enantiomers of deoxydihydroprephenate are substrates for prephenate dehydrogenase,[14] 4 was expected to be oxidized to 6 by the dehydrogenase enzyme in the presence of NAD^+.

Described below are (1) the synthesis of 4, (2) studies of the uncatalyzed and acid-dependant Claisen rearrangement of 4 and the dimethyl ester of 4, and (3) investigations of the enzyme-catalyzed reactions of 4.

The synthesis of 4 is outlined in Scheme III. Diene 7, prepared by a modification of the literature procedure,[15] was oxidized with *m*-chloroperoxybenzoic acid (*m*-CPBA) in CH_2Cl_2 to give 8 in nearly quantitative yield. Reaction of 8 with $PhSe^-$ (Ph_2Se_2, $NaBH_4$) in methanol at 0 °C was regiospecific and gave 9 (73%). Oxidation of 9 with excess 30% H_2O_2 in acetone gave the selenoxide which underwent [2,3] rearrangement to provide 10 (70%).

Protection of the secondary hydroxyl group was accomplished by acylation of 10 with acetic anhydride and triethylamine in methylene chloride to give 11 and 12 in a ratio of 10:1. Product from monoacylation at the tertiary hydroxyl group was not observed. Purification gave pure tertiary alcohol 11(91%) which was converted to 13 by reaction with $MeO_2CC(N_2)PO(OMe)_2/Rh_2(O_2CC_7H_{15})_4$.[16] The lithium salt of 13 was quenched with formaldehyde to give 14 in 30% overall yield from 11.[17] Cleavage of the acetate group of 14 was effected with NaOMe/MeOH to provide 15 (68%). Hydrolysis of 15 with NaOH in THF/H_2O gave 4 which was purified as the disodium salt by ion-exchange chromatography (Bio-Rex 70 sodium ion exchange resin).

In D_2O pD = 7.4 (phosphate buffer) and 30 °C, 4 rearranged quantitatively to 5 (Scheme II) with a half-life of 7.0 h.[18] Enzymatic studies with chorismate mutase–prephenate dehydrogenase from *Escherichia coli*[19] established that 4 was not a substrate for chorismate mutase. Both 4 (K_i = 1.28 mM) and 5 (K_i = 0.97 mM) were moderate competitive inhibitors of chorismate mutase. The implications for the mechanism of chorismate mutase are ambiguous. The binding of 4 is clearly less efficient than that of 1. If binding energies contribute to the acceleration of the mutase reaction, then we would expect 4 to be metabolized more slowly than 1. Furthermore, 4 may not bind to the active site in the optimum geometry for rearrangement, an effect which would further limit the catalytic effectiveness of the enzyme. Thus, without knowing the binding constraints of 4 with chorismate mutase, we cannot extrapolate the results to the mechanistic implications of the enzyme. In the presence of NAD^+, 4 was a reasonable substrate for the prephenate dehydrogenase activity of the enzyme with a V_{max} of 0.16 μmol min^{-1} mg^{-1} compared to prephenate with a V_{max} of 10.0 μmol min^{-1} mg^{-1}. This is not surprising in view of the observation of Cleland and co-workers that the two enantiomers of deoxodihydroprephenate are substrates of prephenate dehydrogenase. One enantiomer had V/K 23-fold higher than the other, and V for the fast isomer was 5% of that of prephenate.[14] The V_{max} observed for 4 presumably is the V_{max} for the enantiomer with the absolute stereochemistry corresponding to the fast reacting enantiomer of deoxodihydroprephenate.

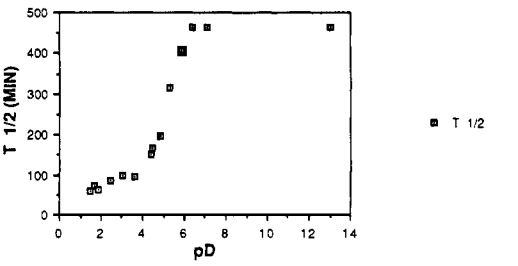
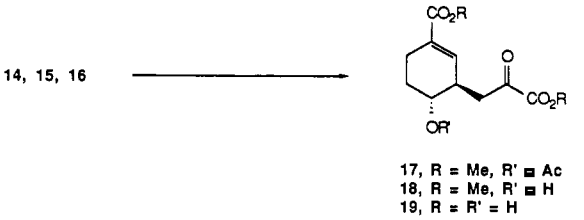


Figure 1. Half-life of rearrangement of 4 versus pD. All data were obtained by ^1H NMR in 0.1M D_2O buffers at 30 °C as follows: pD 1.5 to 3.05; DCl/NaCl. pD 3.6 to 7.0; DOAc/NaOAc. pD 13; NaOD.

Scheme IV



17, R = Me, R' = Ac
18, R = Me, R' = H
19, R = R' = H

Table I. Rate of Claisen Rearrangement of 4 and 14–16

compd	conditions (30 °C)	k, min^{-1}	$T_{1/2}$, h
14	$CDCl_3$	4.6×10^{-4}	25.0
15	$CDCl_3$	7.2×10^{-4}	16.0
15	$CDCl_3$ (20 mol % TFA)	7.6×10^{-4}	15.1
15	MeOD	1.4×10^{-4}	8.2
15	MeOD (15 mol % TsOH)	1.5×10^{-4}	7.7
16 (diacid)	acetone-d_6	5.5×10^{-3}	2.1
4	D_2O (phosphate, pD = 7.4)	1.65×10^{-3}	7.0
4	D_2O (pD = 7.4) + 0.25 M NaCl	1.86×10^{-3}	6.3
4	D_2O (pD = 7.4) + 0.50 M NaCl	2.06×10^{-3}	5.6

Ester derivatives 14 and 15 and 16, the diacid form of 4, underwent quantitative Claisen rearrangement in organic solvents to 17, 18, and 19, respectively (Scheme IV). The half-lives for the rearrangements are listed in Table I. The increased rate of rearrangement of 15 in the more polar solvent MeOD is expected in view of the solvent effects on the rearrangement of chorismate and related compounds.[7,12] Within experimental error no increase in rate of rearrangement of 15 was observed in $CDCl_3$ when trifluoroacetic acid (20 mol %) was added or in MeOD when *p*-toluenesulfonic acid (15 mol %) was added. This lack of acid catalysis for the Claisen rearrangement of 15 was not unexpected since Carpenter and co-workers have reported that no acid catalysis was observed for the Claisen rearrangement of the dimethyl ester of chorismic acid,[12] and Knowles and co-workers have observed the same rate of Claisen rearrangement of chorismic acid in water at pH 7.0 and pH 1.0 (J. R. Knowles, private communication). In contrast, pH dependance was observed for the Claisen rearrangement of 4 in D_2O (Figure 1).[20] The rate of rearrangement was constant above pD = ~6, but the rate

(13) The latter possibility is open to debate.
(14) Hermes, J. D.; Tipton, P. A.; Fisher, M. A.; O'Leary, M. H.; Morrison, J. F.; Cleland, W. W. *Biochemistry* **1984**, *23*, 6263–6275.
(15) Hünig, S.; Kahanek, H. *Chem. Ber.* **1957**, *90*, 238–245.
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(17) After allowing for recovered 11 (36%).
(18) The rate of the uncatalyzed Claisen rearrangement of 1 to 2 is the same in H_2O and D_2O.[7]
(19) Heyde, E.; Morrison, J. F. *Biochemistry* **1978**, *17*, 1573–1580.
(20) As suggested by a reviewer, the data fits a sigmoidal plot (pD vs k) to give pK_a = 4.38 ± 0.07.

increased sharply from pD = 6 to pD = 2. Added salt (NaCl, 0.25 M and 0.5 M) did not greatly affect the rate of rearrangement at pD = 7.4, so ionic effects are a minor factor. Although protonation (deuteriation) of the carboxylate groups of 1 does not affect its rate of rearrangement, protonation of the carboxylate groups is affecting the rate of rearrangement of 4 at low pD.

The sensitivity of 4 to acid may be explained by the substitution pattern of the carboxyl-group on the ring using the Carpenter model.[10] One can conclude from the data for the nitrile-substituted analogues that the C-3 position of allyl vinyl ether is much more sensitive to electronic substituent effects than the C-1 position (approximately 30-fold). Furthermore, C-3 of allyl vinyl ether shows an opposite effect than C-1 upon substitution of an electron-withdrawing substituent. Protonation of carboxylate groups increases the electron-deficient character at C-1, which easily explains the sensitivity of 4 to protonation. Although the rate enhancement in the acid-dependent rearrangement of 4 is small, it is an interesting example of pH dependence of the Claisen rearrangement of a compound related in structure to chorismate.

Experimental Section

^{1}H NMR spectra were obtained at 250 or 300 MHz in $CDCl_3$ and ^{13}C NMR spectra were obtained at 75.45 MHz in $CDCl_3$ with chemical shift values (δ) in parts per million downfield from tetramethylsilane. Microanalyses were performed by Robertson Laboratories, Madison, NJ. Flash chromatography refers to the procedure developed by Still and co-workers.[21] For studies with chorismate mutase, reaction was monitored by disapearance of chorismate (λ = 273 nm, ϵ = 2630).[19] For studies with prephenate dehydrogenase, reaction was performed in the presence of 0.1mM NAD^+ and was monitored by the appearance of NADH (λ = 340 nm, ϵ = 6400). All kinetic studies were performed in 100 mM TRIS·HCl buffer (pH 7.5) with 1 mM EDTA, 1 mM DTT, and 0.1 mg/mL BSA. We thank Dr. Morrison for a generous supply of chorismate mutase–prephenate dehydrogenase.

Methyl 2,3-Dihydrobenzoate (7). Procedure A. To a solution of 3-(diethylamino)-4-carbomethoxycyclohexene[15] (50 g, 237 mmol) in 100 mL of methylene chloride was added methyl iodide (5 equiv, 260 g). The reaction mixture was stirred at room temperature for 21 h. The reaction mixture was concentrated under vacuum, and the residue was dissolved in 300 mL of methylene chloride. The solution was cooled and DBU (2.0 equiv, 72 g) was added dropwise over 10 min. The reaction mixture was stirred at room temperature for 30 min. The solution was washed with 1 N HCl (300 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under vacuum (water aspirator). Vacuum distillation (42–44 °C, 0.3 mmHg) gave 19.3 g (59%) of pure 7 as a colorless oil.

Procedure B. To a solution of 3-(diethylamino)-4-carbomethoxycyclohexene[15] (155 g, 0.734 mol) was added methyl iodide (1.06 equiv, 111 g). The solution was stirred at room temperature for 72 h and diluted with 500 mL of methanol and 500 mL of methylene chloride. NaOH (1.5 equiv, 370 mL of a 3 N solution) was added. The two-phase mixture was stirred vigorously for 10 min. HCl (6 N) was added to bring the solution to pH 4. The layers were separated and the aqueous layer was extracted with methylene chloride (three 600-mL portions). The combined organic fractions were dried ($MgSO_4$), filtered, and concentrated under vacuum (water aspirator). Vacuum distillation yielded 49.2 g. (49%) of pure 7: IR (neat) 3043, 2992, 2950, 2881, 2836, 1711 cm^{-1}; ^{1}H NMR (250 MHz) δ 7.00 (1 H, dd, J = 5.2, 1.9 Hz), 6.12 (2 H, m), 3.77 (3 H, s), 2.46 (2 H, m), 2.27 (2 H, m); ^{13}C NMR δ 167.7, 133.4, 133.1, 127.0, 123.9, 51.5, 22.7, 20.6. Anal. Calcd for $C_8H_{10}O_2$: C, 69.55; H, 7.29. Found: C, 69.54; H, 7.34.

Methyl 7-Oxabicyclo[4.1.0]hept-2-ene-3-carboxylate (8). A solution of 7 (10.0 g, 72.4 mmol) in 250 mL of methylene chloride was cooled to –70 °C. *m*-CPBA (1.1 equiv, 17.2 g) was added and the solution was stirred at –70 °C for 30 min. The solution was allowed to warm to room temperature and was stirred for 2 h. The solution was cooled to –70 °C, filtered, and washed with saturated Na_2SO_3, H_2O, and saturated $NaHCO_3$ (100 mL each). The organic layer was dried (K_2CO_3), filtered, and evaporated to give 11.1 g (99%) of 8 as a light yellow oil.8: IR (neat) 2998, 2954, 2850, 1716, 1437 cm^{-1}; ^{1}H NMR (250 MHz) δ 7.09 (1 H, t, J = 3.6 Hz), 3.74 (3 H, s), 3.58 (1 H, br s), 3.58(1 H, br s), 3.38 (1 H, t, J = 4.1 Hz), 2.56 (1 H, dd, J = 6.3, 1.9 Hz), 2.34 (1 H, dd, J = 15, 8.2 Hz), 2.06 (1 H, m); ^{13}C NMR δ 166.5, 134.1, 133.5, 55.5, 51.8, 46.3, 20.9, 19.7.

Methyl 3β-(Phenylseleno)-4α-hydroxycyclohex-1-ene-1-carboxylate (9). To a stirred solution of diphenyl diselenide (0.75 equiv, 16.9 g, 53.7 mmol) in 500 mL of anhydrous methanol at 0 °C was added portions of $NaBH_4$ until the solution turned colorless. A solution of 8 (11.1 g, 71.7 mmol) in 200 mL of dry methanol was added via cannula. Stirring was continued for 4 h. The solution was evaporated under reduced pressure and the residue was dissolved in 300 mL of CH_2Cl_2. The solution was washed with saturated NH_4Cl solution (200 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was chromatographed on silica gel (1:1 ether/petroleum ether) to give 16.3 g (73%) of pure 9 as a pale yellow oil. 9: IR (neat) 2998, 2954, 2850, 1716, 1437 cm^{-1}; ^{1}H NMR (250 MHz) δ 7.58 (2 H, m), 7.30 (3 H, m), 7.06 (1 H, m), 3.94 (1 H, m), 3.78 (1 H, m), 3.75 (1 H, s), 2.55-1.65 (5 H, m); ^{13}C NMR δ 166.9, 137.0, 134.9, 130.5, 129.2, 128.2, 128.1, 69.3, 51.8, 47.4, 26.6, 21.1; mass spectrum, *m/e* (rel intensity) 312 (15), 310 (4), 234 (1.5), 158 (15), 155 (24), 124 (59), 96 (100).

Methyl 1β,4α-Dihydroxycyclohex-2-ene-1-carboxylate (10). A stirred solution of 9 (16.1 g, 51.8 mmol) in 200 mL of acetone was cooled to 0 °C and H_2O_2 (5 equiv, 8.92 mL) was added dropwise over 5 min. The solution was stirred for 2 h at 0 °C and 24 h at room temperature. The reaction mixture was diluted with ethyl acetate (600 mL), washed with 200 mL of saturated Na_2SO_3 solution, dried ($MgSO_4$), and evaporated. Flash chromatography on silica gel (1:1 ether/petroleum ether) gave 6.27 g (70%) of 10 as a colorless oil. 10: IR (neat) 3377, 3029, 2954, 2872, 1734, 1266 cm^{-1}; ^{1}H NMR (250 MHz) δ 6.05 (1 H, dd, J = 9.4, 3 Hz), 5.68 (1 H, d, J = 9.4 Hz), 4.26 (1 H, br s), 3.82 (3 H, s), 3.6–2.7 (2 H, br s), 2.3–2.05 (2 H, m), 1.9–1.7 (2 H, m); ^{13}C NMR δ 176.0, 134.0, 129.2, 71.6, 64.4, 53.2, 30.9, 28.6; mass spectrum, *m/e* (rel intensity) 172 (0.1), 154 (11), 137 (4), 113 (98), 95 (100); Anal.Calcd for $C_8H_{12}O_4$: C, 55.81; H, 7.02. Found: C, 55.79; H, 7.09.

Methyl 1β-Hydroxy-4α-acetoxycyclohex-2-ene-1-carboxylate (11). Acetic anhydride (1.1 equiv, 3.80 mL) was added dropwise over 5 min to a stirred solution of 10 (6.31 g, 36.6 mmol) and triethylamine (1.5 equiv, 7.65 mL) in 250 mL of CH_2Cl_2 at 0 °C. The solution was allowed to warm to room temperature and was stirred for 20 h. An additional portion of triethylamine (0.8 equiv, 4.1 mL) and acetic anhydride (0.5 equiv, 1.7 mL) was added and stirring was continued for 24 h. The reaction mixture was washed with two 100-mL portions of saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated to give a 10:1 mixture of 11 and 12. Chromatography on silica gel (1:1 ether/petroleum ether) gave 7.11 g of pure 11 (91%) and 760 mg of 12 (8%). 11: IR (neat) 3468, 2955, 1734, 1242 cm^{-1}; ^{1}H NMR (300 MHz) δ 6.02 (1 H, dd, J = 9.4, 4.5 Hz), 5.82 (1 H, d, J = 9.4 Hz), 5.31 (1 H, d, J = 4.5 Hz), 3.83 (3 H, s), 2.3–1.7 (4 H, m), 2.07 (3 H, s); ^{13}C NMR δ 175.9, 170.5, 131.1, 130.0, 71.2, 66.4, 53.2, 30.6, 24.9, 21.2; mass spectrum, *m/e* (rel intensity) 197 (1.2), 155 (20), 154 (19), 137 (9), 96 (33), 95 (100). Anal. Calcd for $C_{10}H_{14}O_5$: C, 56.07; H, 6.58. Found: C, 55.90; H, 6.54. 12: ^{1}H NMR δ 6.30 (1 H, d, J = 10 Hz), 6.03 (1 H, dd, J = 10, 4.5 Hz), 5.28 (1 H, d, J = 4.5 Hz), 3.79 (3 H, s), 2.4–1.8 (4 H, m), 2.08 (3 H, s), 2.06 (3 H, s).

Methyl 1β-[[1-(Methoxycarbonyl)ethenyl]oxy]-4α-acetoxycyclohex-2-ene-1-carboxylate (14). To a solution of 11 (525 mg, 2.45 mmol) in 30 mL of anhydrous benzene was added $Rh_2(n\text{-Oct})_4$ (25 mg) and $MeO_2CC(N_2)PO(OMe)_2$ (1.5 equiv, 760 mg). The solution was heated to 80 °C and stirred for 24 h. Additional $Rh_2(Oct)_4$ (10 mg) and $MeO_2CC(N_2)PO(OMe)_2$ (400 mg) was added and the reaction mixture was stirred at 80 °C for 24 h. The reaction mixture was evaporated and chromatographed on silica gel (9:1 ethyl acetate/petroleum ether) to give 520 mg of crude 13 and 190 mg of recovered 11 (36%). A stirred solution

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of **13** (520 mg, 1.32 mmol) in 12 mL of anhydrous THF was cooled to −65 °C and lithium hexamethyldisilazane (1.00 equiv, 1.32 mL of a 1 N solution) was added via cannula. Stirring was continued at −65 °C for 20 min while formaldehyde, generated by the thermal cracking of paraformaldehyde, was bubbled into the solution. The THF solution was allowed to warm to room temperature and was stirred for 15 min. The THF solution was concentrated under reduced pressure and filtered through a silica plug (1:2 ethyl acetate/petroleum ether) to give 136 mg (30% for two steps) of pure **14** as a colorless oil. **14**: IR (neat) 2955, 1738, 1240 cm^{-1}; ^1H NMR (300 MHz) δ 6.20 (1 H, d, J = 7 Hz), 6.08 (1 H, dd, J = 7, 2.8 Hz), 5.57 (1 H, d, J = 3 Hz), 5.29 (1 H, d, J = 2.8), 4.81 (1 H, d, J = 3 Hz), 3.80 (3 H, s), 3.78 (3 H, s), 2.4–1.8 (4 H, m), 2.05 (3 H, s); ^{13}C NMR δ 171.7, 170.4, 163.7, 147.7, 132.1, 128.2, 102.1, 78.2, 66.4, 52.7, 52.4, 28.4, 24.6, 21.1; mass spectrum, m/e (rel intensity) 298 (0.4), 267 (2.8), 239 (4), 197 (60), 179 (28), 155 (52), 137 (100).

Methyl 1β-[[1-(Methoxycarbonyl)ethenyl]oxy]-4α-hydroxycyclohex-2-ene-1-carboxylate (15). To an ice-cold solution of 14 (65 mg, 0.22 mmol) in 1.5 mL of anhydrous methanol was added NaOMe (3 equiv, 35 mg). The temperature of the reaction mixture was maintained at 0 °C for 1 h. The reaction mixture was diluted with 10 mL of saturated NH_4Cl solution and extracted with three 10-mL portions of CH_2Cl_2. The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and evaporated to give a golden oil which was purified by flash chromatography (1:1 ethyl acetate/petroleum ether) to give 38 mg (68%) of pure **15** as a colorless oil. **15**: IR (neat) 3444, 2955, 1735, 1624, 1439, 1168 cm^{-1}; ^1H NMR (300 MHz) δ 6.13 (1 H, dd, J = 9, 2.8 Hz), 6.08 (1 H, d, J = 9 Hz), 5.57 (1 H, d, J = 3 Hz), 4.70 (1 H, d, J = 3 Hz), 4.27 (1 H,br s), 3.79 (6 H, s), 2.5–1.7 (4 H, m); ^{13}C NMR δ 171.7, 163.5, 147.2, 136.3, 125.8, 101.0, 78.6, 64.5, 52.8, 52.5, 28.5, 28.3; mass spectrum, m/e(rel intensity) 257 (0.7), 256 (5.5), 238 (8.0), 224 (39), 165 (95), 155 (100), 137 (80), 91 (70), 59 (68); HRMS calcd for $C_{12}H_{16}O_6$ 256.0947, found 256.0947.

1β-[(1-Carboxyethenyl)oxy]-4α-hydroxycyclohex-2-ene-1-carboxylic Acid (16) and Disodium Salt 4. To a stirred solution of **15** (87 mg, 0.29 mmol) in 6 mL of THF/H_2O was added NaOH (4 equiv, 1.17 mL of a 1 N solution). Stirring was continued at 0 °C for 1.5 h. The reaction mixture was acidified with HCl (1.2 mL of a 1 N solution) and immediately loaded onto a Bio-Rex 70 sodium ion exchange column (0.5 x 4 in.). Elution with water (50 mL) and lyophilization of the eluent, followed by trituration with anhydrous ether, gave 110 mg (100%) of **4** as a white solid. The diacid form was obtained as follows: a portion of **4** was dissolved in water. The water was acidified with 1 N HCl to pH 3 and extracted with ethyl acetate. The ethyl acetate layer was dried ($MgSO_4$), filtered, and evaporated to give **16** as a colorless oil. **16** (diacid): ^1H NMR (acetone-d_6, 300 MHz) δ 6.18 (1 H, d, J = 11 Hz), 5.95 (1 H, dd, J = 11, 4 Hz), 5.17 (1 H, d, J = 3 Hz), 4.40 (1 H, d, J = 3 Hz), 4.27 (1 H, br s), 2.17 (2 H, m), 1.92 (1 H, m), 1.67 (1 H, m). **4** (disodium salt): ^1H NMR (D_2O, 300 MHz) δ 6.20 (1 H, d, J = 10 Hz), 5.96 (1 H, d, J = 10 Hz), 5.18 (1 H, s), 4.42 (1 H, s), 4.28 (1 H, br s), 2.2–1.6 (4 H, m).

Kinetics of [3,3] Rearrangements of 14, 15, 16, and 4 to 17, 18, and 19. Samples (2.5 mg) of each compound were dissolved in 700 μL of the respective deuteriated solvents in NMR tubes. The samples were maintained at 30 °C either in the NMR probe or in a separate constant-temperature bath. Periodically, ^1H NMR spectra were recorded. K_{rearr} was determined from the slope of the line $\ln [(I_{sm})/(I_{prod} + I_{sm})] = K_{rearr} \times t$, where I_{sm} and I_{prod} represent the integral values of the lowest-field vinyl signal of each species (δ 6.2 and 6.8, respectively). In all cases, the disappearance of starting material obeyed first- order kinetics to at least 90% completion. Results are given in Table I and Figure 1. **17**: ^1H NMR (300 MHz) δ 6.72 (1 H, s), 4.76 (1 H, m), 3.92 (3 H, s), 3.75 (3 H, s), 3.1–2.9 (3 H, m), 2.6–2.2 (2 H, m), 2.06 (3 H, s), 1.9–1.7 (2 H, m). **18**: ^1H NMR (300 MHz) δ 6.68 (1 H, s), 4.29 (1 H, br s), 3.90 (3 H, s), 3.76 (3 H, s), 3.14 (1 H, dd, J = 16, 7.6 Hz), 2.96 (1 H, dd, J = 16, 8 Hz), 2.85 (1 H, m), 2.5–2.1 (2 H, m), 1.9–1.7 (2 H, m). **19** (disodium salt): ^1H NMR (300 MHz) δ 6.78 (1 H, s), 3.63 (1 H, m), 2.96 (1 H, dd, J = 13, 4 Hz), 2.80 (1 H, dd, J = 13, 5.5 Hz), 2.68 (1 H, br s), 2.37 (2 H, m), 1.86 (1 H, m), 1.68 (1 H, m).

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Regiospecific Reduction of the 2-Carbonyl Group of the 6-Hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione System by Selected Metal Hydrides. A Novel Reduction of a Fused Xanthine Nucleus

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A series of substituted 6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones **1** have been reduced to the corresponding 2,3-dihydro-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-diones **2** by treatment with excess lithium borohydride in refluxing dioxane or sodium bis(2-methoxyethoxy)aluminum hydride in a refluxing dimethoxyethane–toluene mixture. The reduction occurs regiospecifically at the carbonyl group at the 2-position of the tricyclic nucleus, as demonstrated by spectroscopic and X-ray crystallographic data. The only side products observed are chromatographically immobile materials. Other reducing agents such as sodium borohydride, borane–tetrahydrofuran, or lithium aluminum hydride fail to effect this reduction. In order to achieve practical reaction rates with lithium borohydride, the solubilities of the substrates have been enhanced by means of silylation. MNDO calculations of the relative stabilities of postulated reaction intermediates suggest a possible explanation of the observed regiospecificity of the reduction.

During the course of preparation of a series of novel pyrimido[2,1-*f*]-purine-2,4,8(1*H*,3*H*,9*H*)-triones[1] with interesting antiinflammatory activity, the 7-(methoxycarbonyl)methyl compound (**1**; Scheme I) was treated with excess lithium borohydride in dioxane. In addition to the desired 7-hydroxyethyl product **2**, a second alcohol was isolated and identified as the 2-desoxy derivative **3**. This selective reduction to the methylene oxidation state of the urea-like carbonyl group of a fused xanthine nucleus in the

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