Stereopopulation Control. III. Facilitation of Intramolecular Conjugate Addition of the Carboxyl Group

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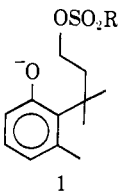
Abstract: The alkylated benzoquinonepropionic acid **3**, 3-(3′,6′-dioxo-2′,4′,5′-trimethylcyclohexa-1′,4′-diene)-3,3-dimethylpropionic acid, while stable in anhydrous solvents, cyclizes in the presence of water to a spirolactone **5**, 4,4,7,8,10-pentamethyl-1-oxaspiro[4.5]dec-7-ene-2,6,9-trione. The pH-dependent equilibrium mixture contains 87% spirolactone at pH 4 and 1% at pH 8. Kinetic analysis (at 30°) shows the ring-opening elimination step to be subject to catalysis by buffer base, proton transfer from **5** to the buffer being the rate-limiting step in the range studied. Conjugate addition involves, at low buffer concentration, a rate-limiting proton transfer from buffer acid to the carbanion of **5**, the cyclic counterpart of the anion of **3**. At moderate buffer concentrations, the rate-limiting dependence on buffer acid disappears as proton transfer becomes faster than the initial addition step. At still higher concentrations of buffer, the rate of lactone formation falls again, for reasons still undetermined. Although the existence of a carbanion species could not be shown by deuterium exchange, the change in rate-determining step makes it a mandatory intermediate in the reaction. The analogous brominated quinonepropionic acid **11**, 3-(3′,6′-dioxo-2′,4′-dibromo-5′-methylcyclohexa-1′,4′-diene)-3,3-dimethylpropionic acid, cyclizes to the spirolactone **12**, 8,10-dibromo-4,4-dimethyl-1-oxaspiro[4.5]dec-7-ene-2,6,9-trione, so rapidly it could not be isolated or even detected under the reaction conditions. Unless 4,4,5-trisubstitution (the trialkyl lock) is present, no conjugate addition can be detected. The unique reactions of **3** and **11** are attributed to the severe conformation-restricting ability of the "lock" and to secondary phenomena resulting therefrom. This reaction provides a useful model for enzyme-catalyzed conjugate addition and elimination.

In preceding papers of this series[2] we have shown how appropriate van der Waals repulsion between nonbonded atoms can be utilized to increase considerably the ground-state populations of conformers highly favorable for the formation of transition states or intermediates. For example, the methyl groups in **1** interlock in such a way (the "trialkyl lock")[3] that the side chain can exist only in a folded (cisoid) conformation; one result is that the specific rate constant for nucleophilic displacement by the phenoxide ion is increased 10^5- to 10^6-fold over that for the nonalkylated analog.[2c] This same set of methyl groups increases the specific rate constant for general acid catalyzed lactonization of **2** over that for its parent (*o*-hydroxyhydrocinnamic



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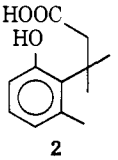

2

acid) by a factor of 10^{10} to 10^{11}.[2b]

In the course of a search for similar rate-enhancement phenomena among other reaction types, we prepared the quinonepropionic acid **3**, in the expectation that the compound would show a preference for its ring tautomer, the hemiketal **4**.[4] A facile ring–chain tautomerism was indeed found, which proved not to be that of **3** ⇌ **4**, but of **3** ⇌ **5**, the result of conjugate addition to one of the quinone double bonds and its reversal. We were prompted to examine this reaction in detail, not only because of its novelty to quinone chemistry, but also because the reversible reaction occurs readily at mild pH and temperature and is very strongly dependent on buffer catalysis in both directions.

Addition of the carboxyl group to activated double bonds is rarely observed and requires strong acid catalysis (lactonization of *cis,cis*-muconic acid)[5a] or an unusually acidic carboxyl group (addition of haloacetic acids to methyl vinyl ketone).[5b] On the other hand, the same muconic acid lactonizes readily at neutral pH with enzymatic catalysis;[6] the present case represents a useful model for the enzymatic reaction and for enzyme-catalyzed conjugate addition in general.[7] Furthermore, the powerful contribution of conformational restriction is made evident by the fact that the addition reaction can be detected *only* in the presence of both the ring and side-chain alkyl groups (the trialkyl lock).

Experimental Section[8]

6-Hydroxy-4,4,5,7,8-pentamethylhydrocoumarin (6) was prepared, in improved yield, by a modification of the method previously described.[2b,9,10] To a solution of 2,3,5-trimethylhydroquinone (15.2 g, 0.1 mol) and methyl 3-methylcrotonate (11.4 g, 0.1 mol) in

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(2) (a) S. Milstien and L. A. Cohen, *Proc. Nat. Acad. Sci. U. S.*, 67, 1143 (1970); (b) S. Milstien and L. A. Cohen, *J. Amer. Chem. Soc.*, **94**, 9158 (1972); (c) R. T. Borchardt and L. A. Cohen, *ibid.*, **94**, 9166 (1972).

(3) Throughout this report, the term "trialkyl lock" will refer to the unique interaction of two alkyl groups at C-4 (in 6) and one alkyl group (or its equivalent) at C-5.

(4) *Cf.* W. Dürckheimer and L. A. Cohen, *J. Amer. Chem. Soc.*, **86**, 4388 (1964).

(5) (a) J. A. Elvidge, R. P. Linstead, B. A. Orkin, P. Sims, H. Baer, and D. B. Pattison, *J. Chem. Soc.*, 2228 (1950); (b) D. Weisleder and M. Friedman, *J. Org. Chem.*, **33**, 3542 (1968).

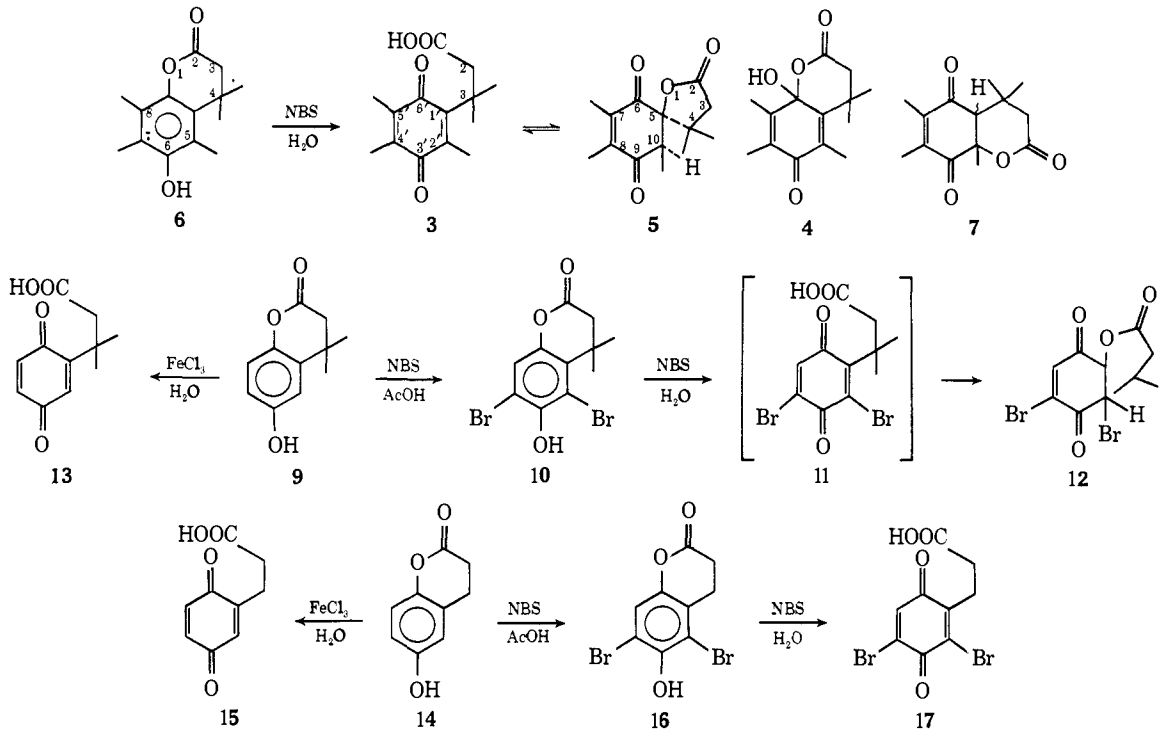
(6) W. R. Sistrom and R. Y. Stanier, *J. Biol. Chem.*, **210**, 821 (1954).

(7) M. Dixon and E. C. Webb, "The Enzymes," Academic Press, New York, N. Y., 1958, p 224.

(8) All analyses were performed by the Analytical Services Section of this laboratory, under the direction of Dr. W. C. Alford. Melting points and boiling points are uncorrected. All compounds were checked for homogeneity by tlc and their mass numbers verified by mass spectroscopy.

(9) S. Milstien and L. A. Cohen, *J. Amer. Chem. Soc.*, **92**, 4377 (1970).

(10) J. Colonge, E. LeSech, and R. Marey, *Bull. Soc. Chim. Fr.*, 776 (1957).



250 ml of benzene was added concentrated sulfuric acid (10.0 g, 0.1 mol). The mixture was heated at reflux for 45 min and then subjected to azeotropic distillation for an additional 45 min. The benzene solution was washed with water, 5% sodium bicarbonate, and saturated brine and dried ($MgSO_4$). Removal of the solvent and crystallization (benzene) afforded 13.6 g (55%) of **6**, mp 186–187° (lit.[2b] mp 186–187°).

3-(3′,6′-Dioxo-2′,4′,5′-trimethylcyclohexa-1′,4′-diene)-3,3-dimethylpropionic Acid (3). To a stirred solution of **6** (1.00 g, 4.27 mmol) in 50 ml of 10% aqueous acetonitrile was added dropwise, at 25°, a solution of *N*-bromosuccinimide (NBS) (0.80 g, 4.5 mmol) in 10 ml of acetonitrile. The reaction mixture was stirred for 1 hr at 25°, diluted with water, and extracted with several portions of ether. The combined ether extracts were washed with water and saturated brine and dried ($MgSO_4$). Removal of solvent and crystallization (acetone–hexane) afforded 1.00 g (94%) of **3**: mp 101–103°; ir ($CHCl_3$) 3430 (OH), 1720 and 1655 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.45 (s, 6 H, C-3 CH_3's), 1.96 (s, 6 H, C-4′ and C-5′ CH_3's), 2.13 (s, 3 H, C-2′ CH_3), and 3.02 ppm (s, 2 H, C-2 CH_2); uv (CH_3OH) λ_{max} 258 nm (ϵ 14,900) and 338 (275).

Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.12; H, 7.27.

Methyl Ester of 3 (8). To a solution of 1.50 g (6.0 mmol) of **3** in 50 ml of methanol was added 1 drop of concentrated sulfuric acid. The mixture was heated at reflux for 5 hr and the solvent removed under reduced pressure. The residual oil was diluted with water and extracted with several portions of ether. The combined ether fractions were washed with water, 5% sodium bicarbonate, and saturated brine and dried ($MgSO_4$). Removal of solvent and distillation afforded 1.20 g (76%) of the ester: bp 125–127° (0.3 mm); ir ($CHCl_3$) 1722 and 1642 cm^{-1} (C=O); nmr (CD_3OD) δ 1.43 (s, 6 H, C-3 CH_3's), 1.95 (s, 6 H, C-4′ and C-5′ CH_3's), 2.13 (s, 3 H, C-2′ CH_3), 2.97 (s, 2 H, C-2 CH_2), and 3.58 ppm (s, 3 H, OCH_3); uv (CH_3OH) λ_{max} 260 nm (ϵ 11,800) and 338 (240).

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.07; H, 7.92.

A solution of **8** in acetate buffer (pH 5.4)–acetonitrile (1:1) showed no spectral change at 338 nm over a 24-hr period.

4,4,7,8,10-Pentamethyl-1-oxaspiro[4.5]-dec-7-ene-2,6,9-trione (5). A solution of 1.0 g (4.0 mmol) of **3** in 100 ml of 0.2 *M* sodium acetate buffer (20% dioxane, pH 4.6) was stirred at 25° for 24 hr; then the mixture was diluted with water and extracted with several portions of ether. The combined ether extracts were washed with 5% sodium bicarbonate, water, and saturated brine and dried ($MgSO_4$). Removal of solvent and crystallization afforded 0.82 g (82%) of the spirolactone **5**: mp 123–126°; ir ($CHCl_3$) 1787 and 1685 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.00 and 1.15 (2 s, 6 H, C-4 CH_3's), 1.39 (d, 3 H, J = 7 Hz, C-10 CH_3), 2.09 (s, 6 H, C-7 and C-8 CH_3's), 2.51 (s, 2 H, C-3 CH_2), and 3.36 ppm (q, 1 H, J = 7 Hz, C-10 CH); uv (CH_3OH) λ_{max} 253 nm (ϵ 12,700) and 338 (89).

Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.21; H, 7.08.

Other reaction conditions were explored for the preparative cyclization of **3**: *e.g.*, methanol, 25° for 20 hr, 6% yield of **5**; 50% aqueous methanol, 25° for 20 hr, 30%; 25% aqueous acetonitrile, 25° for 20 hr, 58%; phosphate buffer (pH 7.0)–methanol (1:1), 25° for 20 hr, 46%. The best yield was obtained by use of the procedure given above. The quinone acid **3** is stable in the presence of 0.1 *N* or 1 *N* hydrochloric acid for at least 24 hr. Storage of **3** for 20 hr in methanol containing 1% trifluoroacetic acid or hydrogen chloride provided only trace amounts of **5** and partial conversion to the methyl ester **8**. No significant cyclization was observed in methanol containing either acetic acid, potassium acetate, or an equimolar mixture of the two.

Reconversion of the Spirolactone 5 into the Quinonepropionic Acid 3. To a solution of 0.05 g (0.2 mmol) of **5** in 5 ml of acetonitrile was added 5 ml of 5% sodium bicarbonate. The reaction mixture immediately turned bright yellow. The mixture was stirred for 3 hr at 25°, diluted with water, acidified to pH 1–2, and extracted with several portions of ether. The combined ether extracts were washed with water and saturated brine and dried ($MgSO_4$). Removal of solvent and crystallization (acetone–hexane) afforded 43 mg (86%) of the acid **3**, mp 101–102°. In methanol containing 0.1% triethylamine, the half-life of the lactone is 10 min. No change in uv spectrum or in tlc behavior of the lactone was observed after 72 hr of storage in 1 *N* hydrochloric acid or in methanol containing 5% hydrogen chloride or 5% trifluoroacetic acid.

5,7-Dibromo-4,4-dimethyl-6-hydroxyhydrocoumarin (10). To a solution of 3.84 g (20 mmol) of **9**[9] in 25 ml of glacial acetic acid was added a solution of NBS (7.12 g, 40 mmol) in 60 ml of warm glacial acetic acid. The reaction mixture was stirred at 50° for 30 min and diluted with 200 ml of water. The mixture was extracted with several portions of ether, and the combined ether fractions were washed with water, 10% sodium carbonate, and saturated brine and dried ($MgSO_4$). Removal of the solvent and chromatography of the resulting oil on silica gel (eluent, 25% ethyl acetate–hexane) afforded 2.20 g of semisolid material. Crystallization from acetone–hexane gave 1.62 g (23%) of the dibromolactone **10**: mp 123–125°; ir ($CHCl_3$) 3500 (OH) and 1770 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.59 (s, 6 H, C-4 CH_3's), 2.67 (s, 2 H, C-3 CH_2), and 6.07 ppm (s, 1 H, C-8 CH).

Anal. Calcd for $C_{11}H_{10}O_3Br_2$: C, 37.75; H, 2.88. Found: C, 38.15; H, 3.03.

Careful examination of the chromatographic fractions failed to reveal any monobrominated material, nor could such species be found following bromination of **9** with 0.5 or 1 equiv of NBS.

8,10-Dibromo-4,4-dimethyl-1-oxaspiro[4.5]dec-7-ene-2,6,9-trione (12). Oxidation of **10** was performed in a manner similar to that for **6**; work-up and crystallization of the residual oil from acetone–hexane provided 0.62 g (60%) of the spirolactone: mp 125–128°; ir ($CHCl_3$) 1809 and 1710 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.31 and 1.46 (2 s, 6 H, C-4 CH_3's), 2.71 and 2.78 (2 s, 2 H, C-3 CH_2), 4.99 (s, 1 H, C-10 CH), and 7.37 ppm (s, 1 H, C-7 CH); uv (CH_3OH) λ_{max} 273 nm (ϵ 6750) and 348 (250).

Anal. Calcd for $C_{11}H_{10}O_4Br_2$: C, 36.10; H, 2.75. Found: C, 36.19; H, 2.39.

No species corresponding to the tautomeric quinonepropionic acid **11** could be found in the mother liquors. Oxidation of **10** in a mixture of acetate buffer (pH 4.6) and acetonitrile (1:1) provided the same results. When the reaction was performed in a uv cell, the spectrum was identical with that of **12** 1 min after addition of NBS; however, in the presence of 2% methanesulfonic acid in aqueous acetonitrile, cyclization of **11** was delayed sufficiently to permit its spectral detection over a 2–4-min period (λ_{max} 288 nm). Efforts to generate **11** by mild alkaline treatment of **12** (as in the analogous **5** to **3** conversion) led only to intensely colored solutions, characteristic of the action of alkali on haloquinones.

3-(3′,6′-Dioxocyclohexa-1′,4′-diene)propionic Acid (15). To a solution of 6-hydroxyhydrocoumarin **14**[11] (1.00 g, 6.1 mmol) in 10 ml of acetonitrile was added 20 ml of 5% ferric chloride hexahydrate in 50% aqueous acetonitrile. The reaction mixture was heated at reflux for 2 hr, diluted with water, and extracted with several portions of ether. The combined ether fractions were washed with water and extracted with three portions of 5% sodium bicarbonate. The combined bicarbonate extracts were acidified with 5% hydrochloric acid, and the acidic solution was extracted with several portions of ether. The combined ether extracts were washed with 5% hydrochloric acid, water, and saturated brine and dried ($MgSO_4$). Removal of the solvent and crystallization (benzene) afforded 0.80 g (73%) of the quinonepropionic acid **15**: mp 139–141° (lit.[12] mp 140.5°); uv (CH_3OH) λ_{max} 252 nm (ϵ 12,050).

3-(3′,6′-Dioxocyclohexa-1′,4′-diene)-3,3-dimethylpropionic Acid (13). Oxidation of the hydroxyhydrocoumarin **9**[9] was performed with ferric chloride as described above for **14**. In this case a reflux time of 30 min was used. Crystallization of the product from benzene gave 58% of **13**: mp 133–136°; ir ($CHCl_3$) 1715 and 1658 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.52 (s, 6 H, C-3 CH_3's) and 3.10 ppm (s, 2 H, C-2 CH_2); uv (CH_3OH) λ_{max} 251 nm (ϵ 10,400).

Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.74; H, 5.80.

3-(2′,4′-Dibromo-3′,6′-dioxocyclohexa-1′,4′-diene)propionic Acid (17). The dibromolactone **16**[11] was oxidized with NBS following the procedure used for **10**. Crystallization of the product from acetone–hexane afforded 81% of **17**: mp 172–175°; ir (KBr) 1702, 1679, and 1655 cm^{-1} (C=O); uv (CH_3OH) λ_{max} 288 nm (ϵ 10,600).

Anal. Calcd for $C_9H_6O_4Br_2$: C, 31.98; H, 1.79. Found: C, 31.69; H, 1.67.

Attempted Cyclization of the Quinonepropionic Acids 13, 15, and 17. Solutions of **13, 15,** and **17** in 1 *M* acetate buffer (pH 4.6)–dioxide (4:1) or in 0.1 *M* phosphate buffer (pH 5.6)–dioxane (4:1) showed no changes in uv spectrum over a 72-hr period, nor did tlc reveal the formation of any new compounds. Heating the same solutions of **13** on a steam bath for 15 min effected no change; the solutions of **15** and **17** underwent partial decomposition with this treatment.

pK_a values were determined by half-neutralization at 30°, in media containing 20% dioxane[13] and μ = 0.2 or 1.0 *M* (KCl): acetic acid, 4.93; phosphate monoanion, 7.17; Tris, 8.10; **3**, 5.16. No significant variation of pK_a with ionic strength was observed in this region. The rate of disappearance of the quinonepropionic acid **3** in the vicinity of pH 5 is sufficiently slow to permit reliable pH measurements.

Equilibrium Measurements. Equilibria were determined over the pH range 3.8–8.7 in various buffer media (30°, μ = 0.2 *M*, 20% dioxane) by measurement of the absorbance at 338 nm. At this wavelength, all three components show maxima but different ϵ values: **5**, ϵ 89; **3**, ϵ 275 (pH 2.8); $\mathbf{3}^-$, ϵ 433 (pH 8.0). The time necessary to achieve equilibrium varied from several minutes at pH 8 to 20 hr at pH 4. The waiting period was significantly reduced at elevated temperatures. From a knowledge of the initial concentration of material, the **3**:$\mathbf{3}^-$ ratio as a function of pH, and the individual ϵ values, the mole fraction of each species present at equilibrium was calculated from the final absorbance value. Equilibrium constants were found to be dependent on pH, and independent of direction of approach to equilibrium, ionic strength (μ = 0.2 or 1.0 *M*), nature, and concentration of buffer. Although the equilibrium constants are independent of buffer concentration, the time required to reach equilibrium decreased markedly with increasing buffer concentration at constant pH.

Kinetic Measurements. Buffers were prepared from commercial, reagent-grade materials, using deionized, distilled water and purified dioxane.[13] All solutions contained 20% (by volume) of dioxane and were maintained at ionic strengths of 0.2 or 1.0 *M* with KCl. The pH of each solution was measured on a Model TTT-1c radiometer pH meter, equipped with scale expander. In selected cases, the pH was measured before and after kinetic runs; in no case was a pH change of greater than 0.02 unit detected.

The rates of cyclization (or decyclization) were measured spectrophotometrically by following the decrease (or increase) in absorbance at 338 nm, using a Model 15 Cary recording spectrophotometer, equipped with scale expander and automatic sample-changing accessory. Constant temperature was maintained by circulation of water from a Haake KT41 constant-temperature bath through the sample holder and walls of the spectrophotometer cell compartment. The temperature in the cell compartment was monitored continuously with a Yellow Springs Telethermometer, whose output was displayed on a 6-in. recorder. All measurements were made at 30 ± 0.05°.

Kinetic runs were initiated by addition of 20 μl of a 0.25 *M* solution of **3** or **5** in dioxane to 2.0 ml of a previously equilibrated solution of buffer in the cuvette. For each run, the change in optical density was recorded continuously until a constant value had been reached. Each run was performed in duplicate or triplicate. First-order rate constants were calculated on a General Electric 265 computer, using a program designed to calculate a least-squares evaluation of a plot of $\ln [A_{inf} - A_0)/(A_{inf} - A_t)]$ *vs.* time. Correlation coefficients were usually greater than 0.9995. Slopes and intercepts of buffer dilution plots were calculated in the same way.

Results and Discussion

The quinonepropionic acids **3**, **11**, and **17** were obtained by oxidation of the respective 6-hydroxyhydrocoumarins with NBS in aqueous acetonitrile;[11a,14] however, preparation of **13** and **15** required the use of a nonhalogen oxidant, such as ferric ion, since the ring positions ortho to the phenolic group are exposed in **9** and in **14**.

The chemical and spectral properties of **3**, in anhydrous media, are those of a normal quinonoid system. The carboxyl group is considered hydrogen bonded to a quinone carbonyl, which assumption is strengthened by the appearance of a single, broad hydroxyl band (3430 cm^{-1}) in the infrared spectrum and by the somewhat exalted pK_a value of 5.16. A gradual loss of ultraviolet spectral absorbance in water-containing media provided the first indication of the instability of **3**.

Exploratory studies showed both the rate and extent of absorbance decrease to be pH dependent, both factors increasing with acidity. The colorless transformation product was readily isolated from preparative runs in various water–solvent mixtures and, optimally (82%), from acetate buffer (pH 4.6) containing 20% dioxane. Since this product appeared to have lost carboxyl acidity and was readily reconverted to the quinonepropionic acid at pH 8–9, it was formulated as a lactone (**5** or **7**). The ultraviolet maximum at 253 nm is consistent with that of an α,β,β-trisubstituted enone chromophore

(11) (a) J. W. Thanassi and L. A. Cohen, *Biochim. Biophys. Acta*, 172, 389 (1969); (b) G. L. Schmir, L. A. Cohen, and B. Witkop, *J. Amer. Chem. Soc.*, 81, 2228 (1959).

(12) M. Asano and T. Kawasaki, *J. Pharm. Soc. Jap.*, 70, 480 (1950).

(13) W. Dasler and C. D. Bauer, *Ind. Eng. Chem., Anal. Ed.*, **18**, 52 (1946).

(14) The halogenated quinonepropionic acid **11** was not isolable.

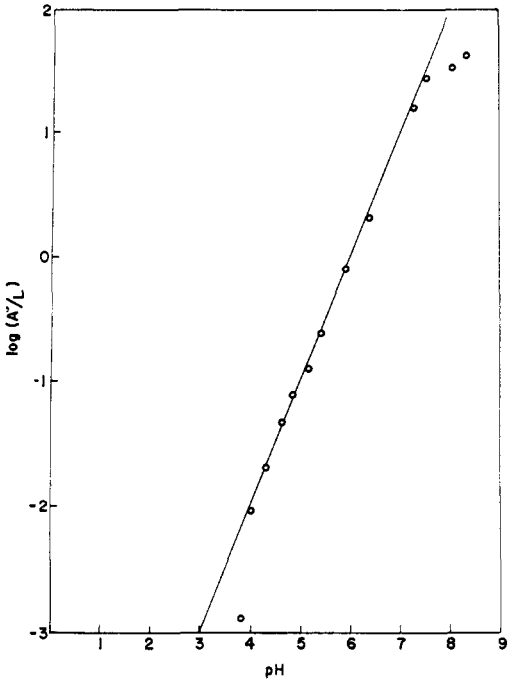


Figure 1. Correlation of equilibria (30°) between **3** and **5** with pH. Plot of log (A^-/L) *vs.* pH according to eq 3.

(calcd, 249 nm);[15] the hydroxydienone **4** would be expected to show an absorption maximum near 240 nm.[4] The nmr spectrum of the lactone clearly shows a symmetrical one-proton quartet (δ 3.36) and a three-proton doublet (δ 1.39), excluding the fused bicyclic structure **7**, as well as **4**. The spirolactone structure is, therefore, most consistent with the spectroscopic data; however, spectroscopy provides little information regarding the stereochemistry of **5**.[16] Examination of space-filling models suggests the least crowded arrangement to be that in which the C(5)–O and the C(10)–H bonds are pseudo-axial, the stereochemistry which would result from normal trans-diaxial addition to the double bond. In this arrangement, one of the C-4 methyl groups is very close to the C-6 oxygen; the difference in chemical shift (0.15 ppm) between the two methyl groups at C-4 may be due to this contrast in environment. A comparison of models of structures **5** and **7** provides no obvious reason, in terms of crowding or strain, for the preference of the spirocyclic over the fused bicyclic system. The conformation of the side chain in **3** is considered cisoid, by virtue of its origin from the lactone ring of **6**, and is restricted to that conformation by the presence of the trialkyl lock.[3] A model of **3** clearly shows that, in this restricted conformation, formation of the transition state leading to **5** is far more likely than that leading to **7**. On the other hand, the formation of **5** may simply reflect a thermodynamic preference of a five- over a six-membered lactone.[17]

While the rates of conversion of **3** to **5**, and *vice versa*, were readily obtainable (see below), the dibromoquinonepropionic acid **11** could neither be isolated nor detected as a transient intermediate under most conditions;[18] conversion of **11** to **12** appeared to be complete within 1–2 min, even in the absence of buffer. No difficulty was observed in effecting the dibromination of **9**, nor could a monobrominated intermediate be detected, even with less than 2 equiv of NBS. Since there appears to be no obstacle to the introduction of bromine at C-5, it is unlikely that the trialkyl lock in **11** (or in **3**) represents a case of steric overcrowding.[19] Since the van der Waals radii of bromine and of methyl are about the same, the more rapid cyclization of **11** over that of **3** may be due to an enhanced reactivity of the bromoquinone double bond. Efforts to regenerate **11** from **12** by mild alkaline treatment led only to intensely colored solutions. These results may be due to the alkaline lability of **11**[20,21] or to that of **12** itself.[22]

That the product of conjugate addition in **11** is also a spirocyclic system (**12**) is evident from the presence of a single proton peak in the nmr spectrum at δ 4.99 (CHBr); a proton peak at this position could not arise from a fused bicyclic structure analogous to **7**. It is interesting to note that the mode of addition in **11** is contrary to electronic considerations; nucleophiles generally replace halogen in haloquinones by an addition–elimination process.[20] On the basis of nmr data, **12** is probably quite similar in stereochemistry and in conformation to **5**. In both cases, the C-4 methyl groups are separated by 0.15 ppm; in the case of **12**, however, the C-3 methylene protons also show a separation of 0.07 ppm. Examination of a model of **12** reveals the proximity of one of these protons to the bromine at C-10, and supports the trans-pseudo-diaxial orientation assigned to both **5** and **12**.

The simpler quinonepropionic acids, **13**, **15**, and **17**, were tested for cyclization ability at pH 4.6 and 5.6. In no case was any spectroscopic change observed over a 72-hr period nor was any new material detected by tlc. The absence of any change in the spectrum of **8** (the methyl ester of **3**) in these buffer systems rules out any competitive bimolecular addition of a nucleophile from the medium.

Acid–Lactone Equilibria (3 ⇌ 5). Essentially complete interconversion between acid **3** (or its anion) and lactone can be observed within the pH range 3.8 (88% lactone) to 8.7 (0.5% lactone). Since the position of equilibrium is pH dependent, eq 1–3 (L is lactone **5** and L its mole fraction; A is acid **3** and A its mole fraction;

$$L \underset{}{\overset{K_1}{\rightleftharpoons}} A^- + H^+ \quad (1)$$

$$K_1 = [(A^-)(H^+)]/L \quad (2)$$

$$\log (A^-/L) = \log K_1 + \text{pH} \quad (3)$$

A^- is 3^- and A^- its mole fraction) were used to establish a relationship between the equilibrium ratios of components and pH. The ratio (A^-/L) was calculated, for each pH value, from spectroscopic measurement of the equilibrium absorbance at 338 nm (see Experimental

(15) L. F. Fieser and M. Fieser, "The Steroids," Reinhold, New York, N. Y., 1959, p 19.

(16) Efforts to clarify the stereochemistries of **5** and **12** by means of the nuclear Overhauser effect provided no conclusive results.

(17) See, however, R. P. Linstead and H. N. Rydon, *J. Chem. Soc.*, 580 (1933).

(18) When **10** was oxidized in aqueous acetonitrile containing 2% methanesulfonic acid, the quinone spectrum (288 nm) was evident for 2–4 min.

(19) This argument is also supported by the facile interconversion of **3** and **5** and by spectroscopic data to be presented in a subsequent report.

(20) J. Miller, "Aromatic Nucleophilic Substitution," Elsevier, New York, N. Y., 1968, p 303.

(21) Compound **17** is also unstable above pH 8.

(22) S. Goldschmidt, H. Zoebelein, and W. Seiz, *Justus Liebigs Ann. Chem.*, 657, 25 (1962).

Section). A plot of log (A^-/L) *vs.* pH provided a line of slope = 1 (Figure 1), as required for strict adherence to eq 3, and a value of log $K_l = -6.02$. Values of (A^-/L) at the intermediate pH values are most accurate, while those at either extreme show weaker correlation owing to the very small concentration of one component. The variation in lactone content of the equilibrium mixture, as a function of pH, is illustrated in Table I. For equilibrium adjustments to kinetic data

Table I. Effect of pH on the **3** ⇌ **5** Equilibrium[a]

pH	Lactone, %	pH	Lactone, %
2.0	88	6.0	48
3.0	88	7.0	9.4
4.0	87	8.0	1.3
5.0	81	9.0	0.2

[a] At 30°, in buffers containing 20% dioxane.

at the extremes of the pH range, values of A^-/L obtained by calculation from eq 4 were used in place of

$$\log(A^-/L) = -6.02 + \text{pH} \qquad (4)$$

the experimental values.

At randomly chosen pH values, the degree of absorbance at equilibrium was found to be essentially independent of direction of approach to equilibrium or ionic strength, or of the nature or concentration of the buffer species. The time required to achieve equilibrium, however, decreased with increase in pH or temperature, or with increase in buffer concentration (at constant pH). The hydroxydienone **4** should exhibit maximum stability in the pH range 5–6[4] and would have negligible absorbance at 338 nm, the wavelength at which equilibrium data were obtained. Since the experimental points in Figure 1 show almost no deviation from the regression line in the pH range 5–6, we conclude that **4** is a negligible component of the equilibrium mixture.

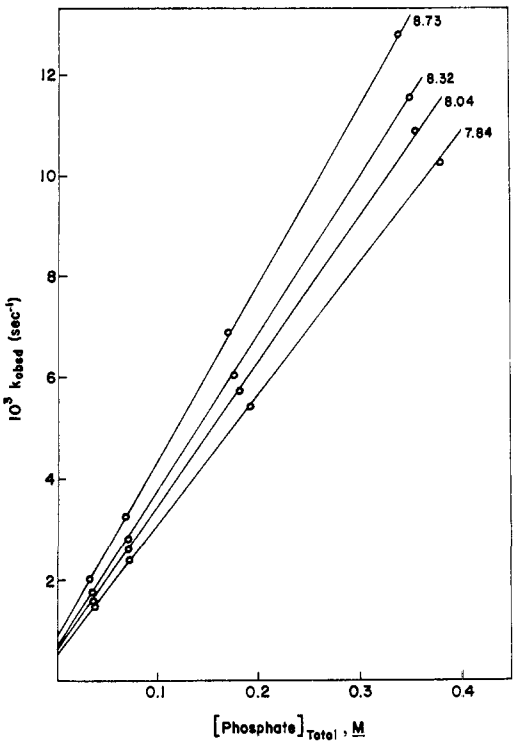


Figure 2. Phosphate buffer catalysis in the conversion of **5** to 3^- at 30° (μ = 0.2 *M*). Plots of k_{obsd} *vs.* $[B]_T$ at the pH values indicated.

(Figure 2) with relatively small intercept values (eq 5).

$$k_{obsd} = (k_l[B] + k_0)(1 + L/A^-) \qquad (5)$$

For each pH, the slope value ($k_{obsd}/[B]_T$) (Table II) was divided by the mole fraction of buffer base (B/B_T) and by a small correction factor for equilibrium ($1 + L/A^-$) to give specific rate constants of 0.132 ± 0.003 M^{-1} sec^{-1} for Tris and 0.033 ± 0.002 M^{-1} sec^{-1} for phosphate. Analysis (eq 6) of the intercept values (k_0) gave

$$k_0 = k_{OH}[OH^-] + k_{H_2O} \qquad (6)$$

Table II. Kinetics of Ring Opening (**5** → **3**)[a]

pH	Buffer	$10^2 k_{obsd}/B_T$,[b] M^{-1} sec^{-1}	B/B_T	$10^2 k_{obsd}/B$,[c] M^{-1} sec^{-1}	$1 + L/A^-$	k_l,[d] M^{-1} sec^{-1}
6.94	Tris	0.95	0.065	14.6	1.115	0.131
7.37	Tris	2.13	0.157	13.6	1.045	0.130
7.96	Tris	5.55	0.420	13.2	1.012	0.130
8.37	Tris	9.10	0.649	14.0	1.004	0.139
						Mean 0.132
7.84	Phos	2.58	0.817	3.2	1.015	0.031
8.04	Phos	2.86	0.876	3.3	1.010	0.032
8.32	Phos	3.10	0.931	3.3	1.005	0.033
8.73	Phos	3.50	0.972	3.6	1.002	0.036
						Mean 0.033

[a] At 30°, μ = 0.2 *M* (KCl), 20% dioxane. [b] Slope values of Figure 2. [c] Slope values/($[B]/[B]_T$). [d] Values of column 5/($1 + L/A^-$).

Kinetics of Ring Opening (5 → 3). Kinetics of conjugate elimination in **5** were measured in Tris (pH 6.9–8.4) and in phosphate (pH 7.8–8.7) buffers. In each run, the reaction was followed to equilibrium (90–99% completion) and was found to obey simple first-order kinetics throughout. Plots of k_{obsd} *vs.* $[B]_T$ (where B = buffer and T signifies total) provided linear slopes a rate constant for specific base, k_{OH}, of about 150 M^{-1} sec^{-1} and for solvent, k_{H_2O}, of about 5.5 × 10^{-4} sec^{-1}. These four values provide a Brønsted slope, β, of about 0.4.

The reaction may be formulated with a rate-limiting loss of the ionizable proton in L, followed by a rapid collapse of L^- (the carbanion of L) to A^- (eq 7), *i.e.*,

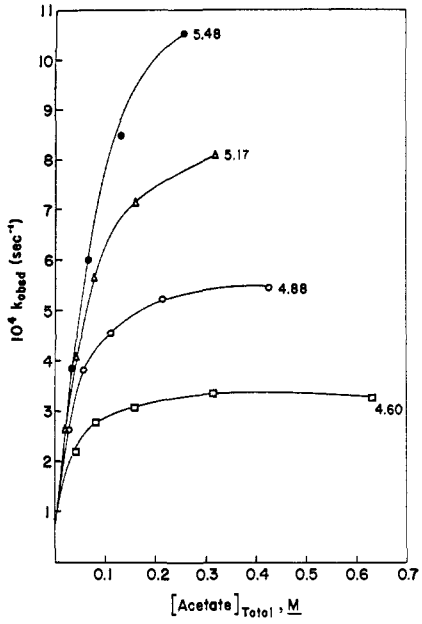


Figure 3. Acetate buffer catalysis in the conversion of **3** to **5** at 30° (μ = 0.2 *M*). Plots of k_{obsd} *vs.* [B]$_T$ at the pH values indicated.

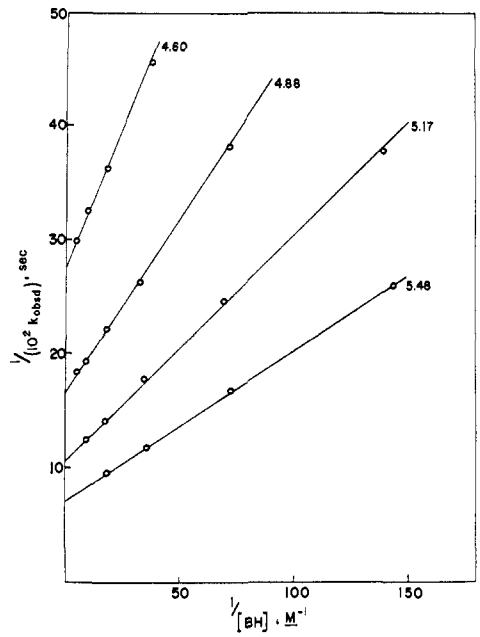
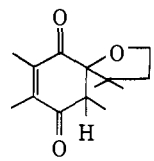


Figure 4. Double reciprocal plots of the data in Figure 3, according to eq 8.

$$L \underset{k_2(BH)}{\overset{k_1(B)}{\rightleftharpoons}} L^- \underset{k_4}{\overset{k_3}{\rightleftharpoons}} A^- \underset{-H^+}{\overset{H^+}{\rightleftharpoons}} A \qquad (7)$$

$k_1(B) < k_3$. As will become evident in the following section, the assumption of a transient L^- species (E1cB) is to be preferred to the kinetically equivalent concerted elimination (E2). No distinction has been made between a carbanion and the canonically equivalent enolate ion formulation for L^-. Ring opening of **5** occurs with comparable speed in methanol containing 0.1% triethylamine ($t_{1/2}$ = 10 min). As is already evident from the equilibrium data, the spirolactone is stable in acidic media. An effort was made to displace the equilibrium by trapping **3** as its methyl ester; however, solutions of **5** in methanol containing hydrogen chloride or trifluoroacetic acid remained unaltered over several days.

Because of the instability of **5** in alkaline media (in Tris buffer, 0.2 *M*, pH 8.4, the lactone has a half-life of *ca.* 6 min), and because of its regeneration from **3**, the ease of exchange of the C-10 proton in the spirolactone could not be evaluated. A solution of **5** in D_2O–CD_3CN (1:1) showed no loss of this proton signal in its nmr spectrum over several hours.[23] Furthermore, **5** was recovered unchanged from efforts to prepare an enol acetate with acetic anhydride–sodium acetate. As an alternative approach, lability of the C-10 hydrogen was examined in the much more stable spiroether **18**.[24] No exchange was detected (by nmr) over 2 days



18

at pH 8–9; in 0.4 *M* sodium deuteroxide, exchange was instantaneous. Unfortunately, efforts to measure the kinetics of exchange in **18** were hampered by a variety of decomposition pathways in strongly alkaline media.

Kinetics of Ring Closure (3 → 5). Kinetics of conjugate addition in **3** were measured in acetate buffer (μ = 0.2 *M*, pH 4.6–5.5); once again the reaction was followed to equilibrium (77–85% completion) and was found to obey simple first-order kinetics. In contrast to the results obtained for ring opening, plots of k_{obsd} *vs.* [B]$_T$ for ring closure were not linear (Figure 3). The trend toward a zero-order dependence on buffer, suggested by these curves, indicates a change in rate-determining step with variation in buffer concentration[25] and requires the existence of an intermediate. According to the principle of microscopic reversibility, it seemed reasonable to assume that the ring-closure reaction is simply the reverse of ring opening formulated in eq 7. On the assumption that the concentration of L^- is always small and that $k_1(B)$ could be neglected, the steady-state approximation provides the double reciprocal eq 8. Thus, plots of $1/k_{obsd}$ *vs.* 1/[BH] pro-

$$\frac{1}{k_{obsd}} = \left[\frac{k_3}{k_2k_4}\left(\frac{1}{[BH]}\right) + \frac{1}{k_4}\right] \times \left[\left(\frac{1}{A^-}\right)\left(\frac{1}{1 + A^-/L}\right)\right] \qquad (8)$$

vided the straight lines of Figure 4, whose slopes and intercepts are recorded in Table III.[26] These values were multiplied by the mole fraction of **3** present in the anionic form (A^-) and by ($1 + A^-/L$) to correct for equilibrium, giving mean values of $k_4 = 1.62 \times 10^{-3}$ sec^{-1} and $k_2/k_3 = 54.7\ M^{-1}$ (Table III). Since eq 7 can be rewritten as eq 9, and since the equilibrium ratios

$$\frac{k_1k_3}{k_2k_4}\left(\frac{[B]}{[BH]}\right) = \frac{A^-}{L} \qquad (9)$$

(23) *Cf.* T. I. Crowell, R. T. Kemp, R. E. Lutz, and A. A. Wall, *J. Amer. Chem. Soc.*, 90, 4638 (1968).
(24) R. T. Borchardt and L. A. Cohen, manuscript in preparation.
(25) For a similar case, see L. R. Fedor and W. R. Glave, *J. Amer. Chem. Soc.*, 93, 985 (1971).
(26) The assumption is made, and is supported by the consistency of the results, that the contributions to k_{obsd} of hydronium ion or of solvent-catalyzed cyclization are negligible.

Table III. Kinetics of Ring Closure at $\mu = 0.2\ M$ (3 → 5)[a]

pH	A^-	$1 + A^-/L$	Slopes,[b] M sec	Intercepts,[b] 10^{-2} sec	k_3/k_2k_4,[c] M sec	10^3k_4,[d] sec^{-1}	k_2/k_3, M^{-1}
4.60	0.216	1.038	50.0	27.5	11.21	1.62	55.0
4.88	0.344	1.072	30.2	16.7	11.11	1.62	55.5
5.17	0.506	1.141	19.7	10.7	11.39	1.62	54.2
5.48	0.676	1.288	13.1	7.1	11.40	1.62	54.2
						Mean 1.62	54.7

[a] At 30° in acetate buffer containing 20% dioxane. [b] Taken from the data of Figure 4. [c] Slopes × (A^-) × $(1 + A^-/L)$. [d] The reciprocals of [intercepts × (A^-) × $(1 + A^-/L)$].

(A^-/L) had been determined previously, k_1 for acetate ion could be calculated. A mean value of 7.2×10^{-3} M^{-1} sec^{-1} was obtained, in agreement with that predicted from the β value of 0.4.

The kinetic analysis suggests a relatively rapid cyclization of A^- to L^-. At low concentrations of buffer acid,[27] proton transfer to L^- is rate limiting with a first-order dependence on [BH]; as [BH] increases, however, proton transfer becomes so rapid that the rate of formation of L^- from A^- becomes rate limiting. Although the buffer acid still functions to convert L^- to L, its kinetic order has been reduced to zero. Thus, the flat portions of the lower curves in Figure 3 should, when extrapolated to zero buffer concentration, provide approximate values of $k_4(A^-)$, as indeed they do. In principle, the same effect should be observed in the ring-opening reaction; that is, at sufficiently high concentrations of buffer base, the slopes of Figure 2 should begin to curve and k_{obsd} should become independent of buffer concentration.[25] The ring-opening reaction, however, was not studied at sufficiently high buffer concentrations to observe this effect.

The curve for pH 4.6 in Figure 3 appears to reverse direction as $[B]_T$ continues to increase. In order to explore this phenomenon more fully, cyclization kinetics were reexamined at a higher ionic strength (μ = 1.0 M) and still higher buffer concentrations, with the results shown in Figure 5. It would appear that concentrations of acetic acid (or of acetate ion) beyond those recorded in Figure 3 inhibit cyclization or effect its reversal.

Initially, we considered that the rate reversal might be due to loss of acetic acid by dimerization at these relatively high concentrations. If acetic acid dimer is viewed as a cyclic species in aqueous media,[28] and catalytically inert, approximate calculations indicate that this factor could only account for a small fraction of the decrease in k_{obsd} actually found. If acetic acid dimer, in aqueous solution, is viewed as a linear species,[29] the loss of activity should be even less significant, since the linear dimer is stated to be a stronger acid than acetic acid monomer; consequently, the dimer should be a more effective general acid and compensation should nullify the effect of dimerization. Since all kinetics were measured in media containing 20% dioxane, it is unlikely that the additional effect of 1–2 M acetic acid on dielectric properties could be significant.

Alternative explanations might be based on deactivation of A or A^- by virtue of its complexation with a buffer component. The linearity of the plot in Figure 1 does not support this explanation; however, equilibrium measurements were made at much lower buffer concentrations. Assuming that the addition and elimination steps follow the same mechanistic pathway over the entire buffer range, we are, at present, unable to offer a definite explanation for this effect.

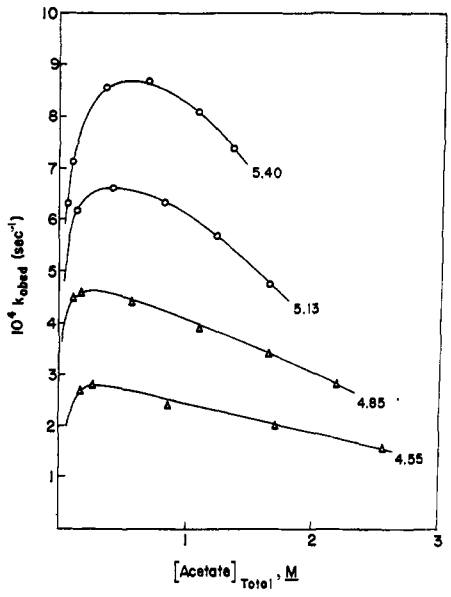


Figure 5. Acetate buffer catalysis in the conversion of 3 to 5 at 30° (μ = 1.0 M). Plots of k_{obsd} *vs.* $[B]_T$ at the pH values indicated.

As seen from the kinetic analysis, effective cyclization requires the presence of 3 in its anion form and the buffer species in its protonated form. In more normal cases, this combination would lead to a bell-shaped plot of k_{obsd} *vs.* pH and constant buffer concentration. Despite the fact that equilibrium data show 5 to be highly favored at low pH, the failure of 3 to cyclize in strongly acidic media becomes intelligible. Furthermore, the stability of the quinonepropionic acid in nonaqueous solvents, in which ionization of the carboxyl group would be repressed, can now be rationalized. The variable yields of 5 realized in different water–solvent mixtures are probably due to the slow approach to equilibrium in these buffer-free media, rather than to any marked dependence of the equilibrium constant on solvent polarity.

Conclusions

Introduction of a "trialkyl lock" into a quinonepropionic acid has provided a useful model for enzyme-catalyzed conjugate addition and elimination. The

(27) At these low buffer concentrations, the BH:3 ratio ranges from 2–8.

(28) A. Katchalsky, H. Eisenberg, and S. Lifson, *J. Amer. Chem. Soc.*, 73, 5889 (1951).

(29) D. L. Martin and F. J. C. Rossotti, *Proc. Chem. Soc., London*, 73 (1961).

two extremes can be almost fully realized within the pH range 4–8, at 30°, and with an overwhelming dependence on buffer catalysis in both directions. Since a comparable reaction could not be detected in the absence of the *complete* lock, it is impossible to make rate enhancement or equilibrium enhancement comparisons, as was done in our earlier studies. As emphasized in the preceding papers,[2] the overall effect of rate or equilibrium enhancement should be attributed not to the loss of rotational freedom alone, but also to a combination of secondary consequences, which may include improvement in orientation, reduced solvation, interorbital distortion and penetration, and perhaps other phenomena not yet recognized. The overall result is a considerable diminution of the free energy barrier between ground and transition states.

Acknowledgment. We are indebted to Drs. L. R. Fedor, W. P. Jencks, G. L. Schmir, and R. L. Schowen for valuable comments and criticisms.

Correlation of Reaction Rate Acceleration with Rotational Restriction. Crystal-Structure Analysis of Compounds with a Trialkyl Lock

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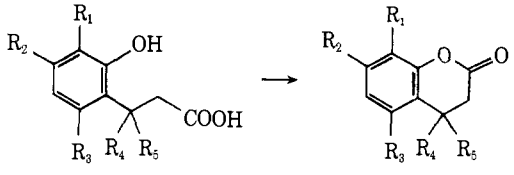
Abstract: Enzyme-promoted reactions have a rate advantage of 10^{11}–10^{15} over their nonenzymatic counterparts. The enzyme is believed to associate closely with the substrate in such a manner as to restrict the conformation of the substrate to a geometry favorable for the reaction to take place. A similar enhancement of reaction rate of lactonization in *o*-hydroxyhydrocinnamic acid has been achieved by substitution of methyl groups both on the ring and the side chain (Milstien and Cohen). Crystal-structure analyses of the "over-methylated" lactone pentamethylhydrocoumarin [space group $P2_1$ (disordered); a = 10.148, b = 8.766, and c = 7.802 Å; β = 115.45°] and the alcohol analog of pentamethyl-*o*-hydroxyhydrocinnamic acid [space group $C\bar{1}$; four independent molecules per asymmetric unit; a = 11.615, b = 25.987, and c = 18.150 Å; α = 93.66°; β = 90.38°; γ = 98.36°] show that the conformation of the acid has been restricted by the presence of the "trialkyl lock" to be similar to that of the lactone. The four independent molecules of the alcohol analog of the acid are almost identical in bond lengths and angles and do not display rotational isomerism. Large angular distortions occur within and without the aromatic ring in order to accommodate the overmethylation.

The great enhancement of the reaction rate of a substrate promoted by an enzyme suggests that the enzyme may impose conformational restrictions on the substrate. Both Jencks and Bruice support the view that approximation of reactants in a rigid system provides *via* an entropy factor the type of rate enhancement desired for enzymatic reactions.[1] Such restriction of the rotational freedom of a substrate by an enzyme, with the accompanying decrease of rotational entropy, may be comparable to chemical alteration of an organic compound, *e.g.*, by alkylation, in order to narrow its conformational range. A study has been performed by Milstien and Cohen[2] on the rate of lactonization of *o*-hydroxyhydrocinnamic acid and its methyl derivatives. The substitution of CH_3 groups for H at the sites R_1 to R_5 enhances the rate of reaction by factors up to 10^{11} as shown in Table I. Milstien and Cohen[2] proposed that the interlocking of the CH_3 group at R_3 with the two CH_3 groups on the side chain at R_4 and R_5 restricted the acid group to the proximity of the OH group and thus was a major factor in promoting the rapid lactonization. To confirm this proposal as well as to establish the precise conformations of these highly methylated compounds, a crystal-structure analysis was undertaken. The very reactive acid IV is too unstable for an X-ray diffraction analysis, hence a crystal of the alcohol analog VI was used.[3] In addition, the crystal structure of the lactone V with R_1–$R_5 \equiv CH_3$ was established.



I–IV V

Table I. Specific Rate Constants[a] for Lactonization (M^{-1} sec^{-1})

Alkyl substitution	$K'_{H_3O^+}$ [b]	Rel rate
I, R_1–$R_5 \equiv H$	5.9×10^{-6}	1.0
II, R_1–$R_3 \equiv CH_3$	4.0×10^{-5}	6.7
III, R_4–$R_5 \equiv CH_3$	2.6×10^{-2}	4.4×10^3
IV, R_1–$R_5 \equiv CH_3$	2.0×10^{6}	3.4×10^{11}

[a] Reference 2. [b] At 30°, in 20% dioxane, μ = 0.3 *M*.

(1) See, *e.g.*, D. F. Koshland, K. W. Carraway, G. A. Dafforn, J. D. Gass, and D. R. Storm, *Cold Spring Harbor Symp. Quant. Biol.*, 36, 13 (1972); T. C. Bruice, *ibid.*, 36, 21 (1972); W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969.

(2) S. Milstien and L. A. Cohen, *Proc. Nat. Acad. Sci. U. S.*, 67, 1143 (1970); *J. Amer. Chem. Soc.*, 94, 9158 (1972).

(3) R. T. Borchardt and L. A. Cohen, *J. Amer. Chem. Soc.*, 94, 9166, 9175 (1972).