Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.64; H, 7.99. Found: C, 66.71; H, 7.72.

When a solution of **15** in aqueous methanol is aerated for 1–2 hr, the quinone **14** is regenerated; under the same conditions, **10a** shows no tendency to undergo air oxidation. When a solution of **15** in tetrahydrofuran is heated at reflux for 2 hr with lithium aluminum hydride, the principal product present, according to tlc, is **10a**, only a trace of **15** remaining; however, when a solution of **15** in ether is shaken with aqueous sodium hydrosulfite, no conversion to **10a** can be detected. Equivalent amounts of **15** and **6** were dissolved in 10 ml of acetonitrile and the solution was diluted with 10 ml of 0.1 *N* hydrochloric acid. After 15 hr, tlc showed **14** and **5** to be the major products present (in roughly equal amount), with only traces of the initial components.

Test for Proton Exchange in 16. Solutions of the quinone **16**[2] in CD_3OD, 0.5 *N* in deuterium chloride or $NaOCD_3$, were examined by nmr over a 24-hr period at 30–35°; there was no significant spectral change in either solution. The same solutions were maintained at 50–55° for 1 hr; again there was no evidence for exchange at the methyl groups.

Conversion of 6 to 4. A sample of **6**, which had been stored in the crystalline state (in a capped vial) for approximately 1 month was observed to have undergone a transformation, resulting in contamination with a colorless, more polar substance. Separation of 0.20 g of the mixture on silica gel (eluent, 25% ethyl acetate–hexane) afforded, after crystallization from acetone–hexane, 0.046 g (24%) of **4**, mp 185–187°. Identity with an authentic sample was confirmed by means of ir and nmr spectra, and mixture melting point. After 3 months, the same sample consisted almost entirely of **4**, as determined by tlc and uv spectral analysis. A solution of **6**, freshly prepared, in 80% aqueous methanol showed no loss of the quinone chromophore over 3 days at 25°. Solutions of **6** in acetate buffer (0.2 *M*, pH 4 or 5)–methanol (4:1) showed a loss of 50–75% of the quinone chromophore in the same 3-day period; the presence of **4** in these reaction mixtures was demonstrated by tlc.

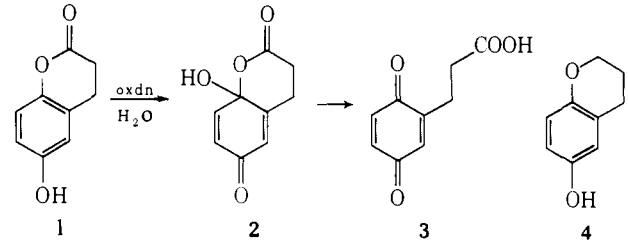
Stereopopulation Control. VI. Conformational Selection of Alternative Oxidation Pathways

Ronald T. Borchardt and Louis A. Cohen*

Contribution from the Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014. *Received June 22, 1973*

Abstract: Aqueous oxidation of 6-hydroxy-4,4,5,7,8-pentamethylhydrocoumarin (**5**) or of 5,7-dibromo-4,4-dimethyl-6-hydroxyhydrocoumarin (**7**) results in the formation of the corresponding quinonepropionic acids. In the presence of $H_2{}^{18}O$, isotopic oxygen is found only in the quinone carbonyl, implicating a *p*-hydroxydienone intermediate in the oxidation pathway. Under the same conditions, the oxidation of 5,7-dibromo-6-hydroxyhydrocoumarin (**10**) provides a quinonepropionic acid with label only in the carboxyl group. In this case, an acylium ion intermediate is proposed. In ethanol as solvent, all three hydrocoumarins form *p*-ethoxydienones on oxidation; in the case of **10**, an equivalent amount of the quinonepropionic ester is also formed; in this reaction, both pathways are followed competitively. In the presence of acetate ion, **5** and **7** form *p*-acetoxydienones while **10** forms a mixed anhydride between acetic acid and the quinonepropionic acid. The *p*-acetoxydienone of **5** rearranges to the thermodynamically preferable *o*-acetoxydienone *via* an SN2′ displacement with acetate ion as nucleophile. The reaction is considerably accelerated upon addition of acetic acid. With acetic acid alone, the *p*-acetoxydienone of **5** rearranges to a quinone mixed anhydride, as does that of **7**. The *p*-acetoxydienone of **7**, however, is not converted into an *o*-acetoxydienone in acetic acid–sodium acetate. Toward aqueous acid hydrolysis, the *p*-ethoxydienone of **10** is more labile than that of **7** by a factor of 5300. The results of labeling in $H_2{}^{18}O$, the differences in oxidation pathways, and the differences in stability to hydrolysis are all attributed to the influence of stereopopulation control. The 4,4,5 trisubstitution in **5** and **7** represses formation of the acylium ion which would lead to quinone esters and anhydrides and, instead, promotes reactions of the cyclic carbonium ion, leading to para-substituted dienones.

Oxidation of 6-hydroxyhydrocoumarins **1**[1] in water-containing media proceeds, as expected, to form the corresponding quinonepropionic acids **3**.[2] Generally, the quinone acids are obtained in good yield and are free of significant side products.[3] It is logical to assume that oxidations of all 6-hydroxyhydrocoumarins follow a common mechanism, involving the thermodynamically unstable *p*-hydroxydienone **2**. The assumption of this intermediate is based on the demonstration of a parallel pathway in the oxidation of 6-chromanols **4**, since their moderately stable *p*-hydroxydienones have been isolated in three cases.[4] In the course of our studies on stereopopulation control[2a] and on conservation of oxidative free energy[5] in such systems, it became apparent that the assumption of a



1 2 3 4

(1) Structures **1–4** are intended to represent general classes of compounds, of which they are the least substituted members.

(2) (a) R. T. Borchardt and L. A. Cohen, *J. Amer. Chem. Soc.*, **94**, 9175 (1972); (b) M. Asano and T. Kawasaki, *J. Pharm. Soc. Jap.*, 70, 480 (1950); (c) L. I. Smith and R. O. Denyes, *J. Amer. Chem. Soc.*, **58**, 304 (1936).

(3) This generalization ignores the fact that certain members of series **3** are unstable,[2a] and are consumed in subsequent transformations.

(4) (a) R. T. Borchardt and L. A. Cohen, *J. Amer. Chem. Soc.*, **95**, 8308 (1973); (b) W. Dürckheimer and L. A. Cohen, *ibid.*, **86**, 4388 (1964); (c) N. I. Bruckner and N. L. Bauld, *J. Org. Chem.*, **36**, 4045 (1971).

(5) J. W. Thanassi and L. A. Cohen, *J. Amer. Chem. Soc.*, **89**, 5733 (1967); *Biochim. Biophys. Acta*, **172**, 389 (1969).

single mechanism for the conversion of **1** to **3** may be invalid, and that any parallelism in the chemistries of **1** and **4** is of limited scope. A duality of pathways in the oxidation of 6-hydroxyhydrocoumarins was first revealed by ^{18}O experiments, the results of which have stimulated a more intensive study of reaction intermediates and their transformation products.

Of a number of representatives of series **1** investigated, three were selected for detailed study. The lactones **5** and **7** belong to the subset containing a trialkyl lock (4,4,5 trisubstitution)[6] and follow the "normal" oxidation pathway *via* **2**; lactone **10** is representative of the large majority of these compounds, and follows an "abnormal" pathway, which does not include **2**.

Results and Discussion

Oxidation of the 6-hydroxyhydrocoumarin **5** with *N*-bromosuccinimide (NBS) in acetonitrile–$H_2{}^{18}O$ provides the quinonepropionic acid **6**, as reported previously.[2a] Exchange of isotope into **6** does not occur readily; the quinone was in contact with $H_2{}^{18}O$ for a total of 7 min during the experiment, and control runs showed that even after 4 hr of contact with $H_2{}^{18}O$, **6** had undergone no detectable exchange.[7] The isotopic enrichment of **6** corresponded to approximately the solvent composition of ^{18}O (0.90 atom); from analysis of the infrared carbonyl bands and the mass spectral fragmentation pattern (see Experimental Section), it was clear that the ^{18}O was contained exclusively in a quinone oxygen, undoubtedly at the site ortho to the propionic acid side chain. This result is entirely consistent with the assumption of an intermediate **5b** and with the general mechanism **1** → **2** → **3**. An analogous experiment with the dibromo derivative **7** provided parallel results. In this case, the quinonepropionic acid **8** is not isolable, and is recovered as its stable spirocyclic isomer **9**.[2a] The isotopic content of **9** was slightly in excess of solvent composition, since random exchange in a haloquinone or ketone is more rapid than in an alkylated quinone.[7a] Infrared and mass spectra again showed all the ^{18}O to be in the quinone and none in the lactone function, this result implicating the intermediate **7b**. When **10**, the simpler analog of **7**, was subjected to oxidation under the same conditions, the quinonepropionic acid **11** was found to contain essentially all its ^{18}O in the carboxyl group and *none* in the quinone carbonyls. As further evidence for these conclusions, **9** lost all its ^{18}O by exchange with normal water, but **11** lost none, these results being consistent with previous experience.[7b] All three lactones, **5**, **7**, and **10**, were also oxidized in deuterium oxide; nmr and mass spectra of the products showed that none had formed any carbon–deuterium bonds[8] and that ketene intermediates, therefore, could be ruled out. There seems no obvious way to explain the labeling pattern in **11** *via* an intermediate analogous to **7b**; tentatively, we propose a pathway involving either a concerted attack by water at the carbonyl of **10a** or, more likely, solvolysis of the acylium ion **10b**.[9]

These results stimulated a search for other ways in which the chemical behavior of **10** might differ from that of **5** or **7**. The NBS oxidations of **5** and of **7** in ethanol provide the respective *p*-ethoxydienones **12** and **21** in high yield. The structures of these products are consistent with expectations based on the $H_2{}^{18}O$

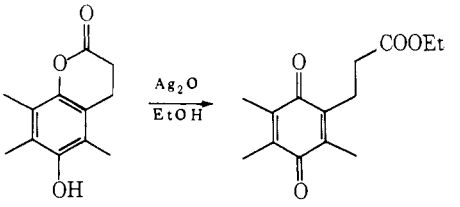
(6) The locking effect of the bromine atom at C-5 is at least as great as that of a methyl group at the same position: ref 2a and S. M. Milstien and L. A. Cohen, *J. Amer Chem. Soc.*, **94**, 9158 (1972).

(7) This result is consistent with the resistance to exchange noted for duroquinone: (a) H. Dahn and J.-D. Aubort, *Helv. Chim. Acta*, **51**, 1348 (1968); (b) D. Samuel and B. L. Silver, *Advan. Phys. Org. Chem.*, **3**, 123 (1965).

(8) The spirolactone **9** contained one exchangeable deuterium atom adjacent to the ketonic carbonyl group (ref 2a).

(9) (a) Although we have no data to exclude a tetrahedral intermediate, the rapidity of the conversion of **10** to **11** makes an addition–elimination mechanism seem less likely. (b) Whether these carbonium ions are viewed as canonical forms of a resonance hybrid or as distinct species in equilibrium has little effect on our subsequent arguments.

results above and with the ultraviolet spectral properties of *p*-ethoxydienones derived from 6-chromanols (**4**), *e.g.*, those of α-tocopherol[4b,10] and of the chromanol analog of **5**.[11,12] Under the same reaction conditions, **10** provided 50% of its *p*-ethoxydienone **24** and 50% of **25**, the ethyl ester of **11**.[13] The ratio of these products did not change with the waiting period prior to work-up, nor could any mild conditions be found for the conversion of **24** into **25**. The isolation of **25** supports the argument for dual pathways, this product evidently having been formed by ethanolysis of **10b**;[14] the formation of **24** demonstrates that **10** can also react by the "normal" pathway and that the partitioning of pathways may be determined by the nucleophile or Lewis base involved.[15] Interestingly, the oxidation of **19** (with silver oxide in ethanol) to the quinone ester **20**



19 20

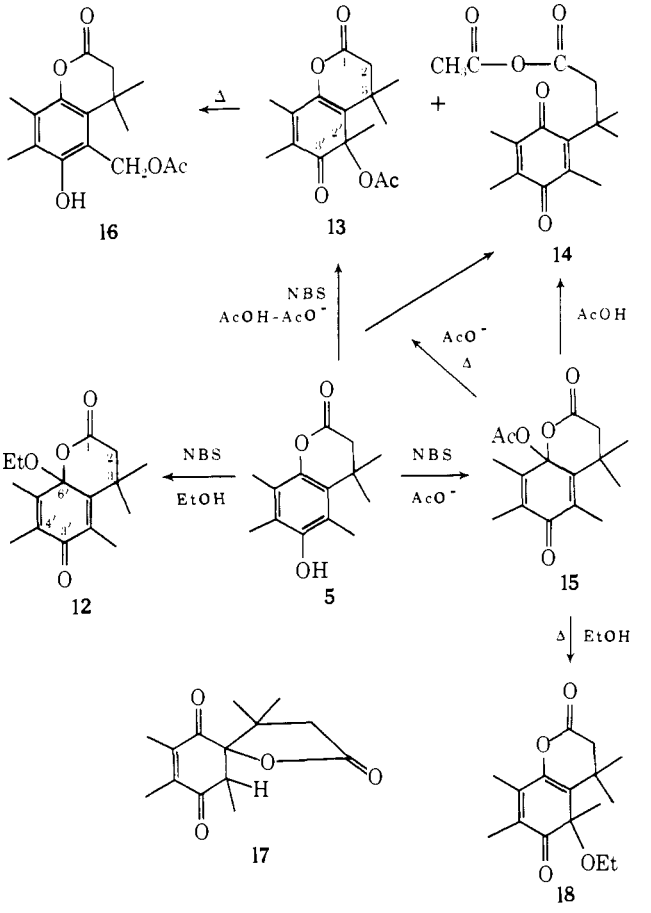
was reported in 1939;[16] the novelty of this observation seems to have escaped notice.

Oxidation of **5** with NBS in a mixture of anhydrous acetic acid and sodium acetate provided, after aqueous work-up, a 78% yield of the *o*-acetoxydienone **13** and 16% of the acid **6** (Scheme I). Several methods were used to demonstrate that **6** was not an immediate product of the reaction, but arose *via* hydrolysis of the mixed anhydride **14**: (1) treatment of the original reaction mixture with *p*-bromoaniline in place of water resulted in the same yield of **13** and 7% of *p*-bromoacetanilide;[5,17,18] (2) in this reaction medium, **6** undergoes slow cyclization to an equilibrium mixture with its spirolactone tautomer **17**; however, the spectral change associated with this transformation was not observed in the reaction mixture following oxidation; (3) the acid anhydride content of the reaction mixture, assayed spectroscopically with ethyl *p*-aminocinnamate (see Experimental Section), corresponded to 16–18%.

The failure to detect **17**, or to lose anhydride material during lyophilization also shows that **14** is not in facile equilibrium with acetic anhydride.

The oxidation of **7** under the same conditions provided yields of quinone anhydride **23** and acetoxy-

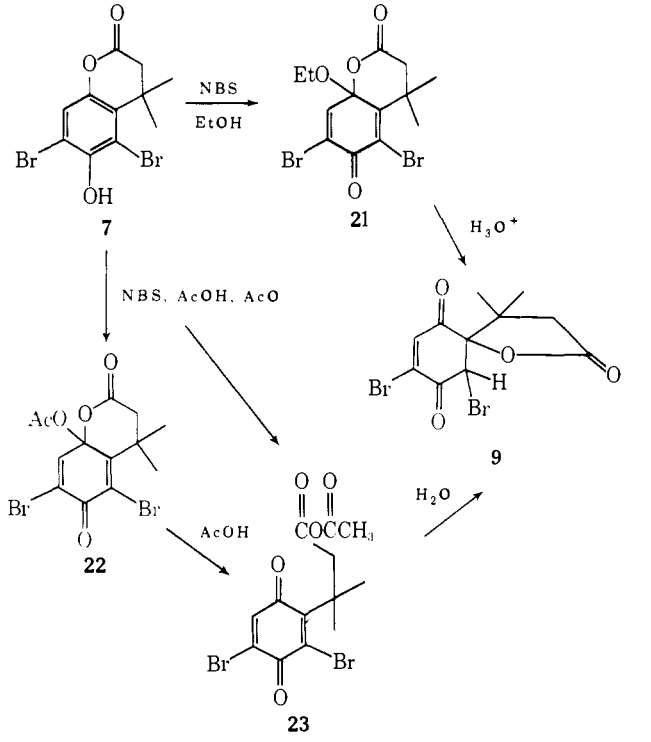
Scheme I



16 13 14 12 5 15 17 18

dienone comparable to those obtained from **5**, except that this dienone proved to be the para isomer **22** (Scheme II). The arguments presented above for the

Scheme II



7 21 9 22 23

formation of **14** (and not **6**) are even stronger in the case of **23** *vs.* **8**, since the latter species cyclizes to **9** in less than 5 min. An analogous oxidation of **10**

(10) C. Martius and H. Eilingsfeld, *Justus Liebigs Ann. Chem.*, 607, 159 (1957); P. D. Boyer, *J. Amer. Chem. Soc.*, 73, 733 (1951).

(11) L. A. Cohen, unpublished observations.

(12) The ultraviolet spectrum of 21 corresponds to that of the dimethylketal of 2,6-dibromoquinone: see W. Dürckheimer and L. A. Cohen, *Biochemistry*, 3, 1948 (1964).

(13) Under these reaction conditions, **11** shows almost no tendency to undergo esterification.

(14) Quinonepropionic esters could not be detected as contaminants of **12** and **21**.

(15) The ratio of products may also vary with the nature of the oxidant and the mechanism of its action; these possibilities are under investigation.

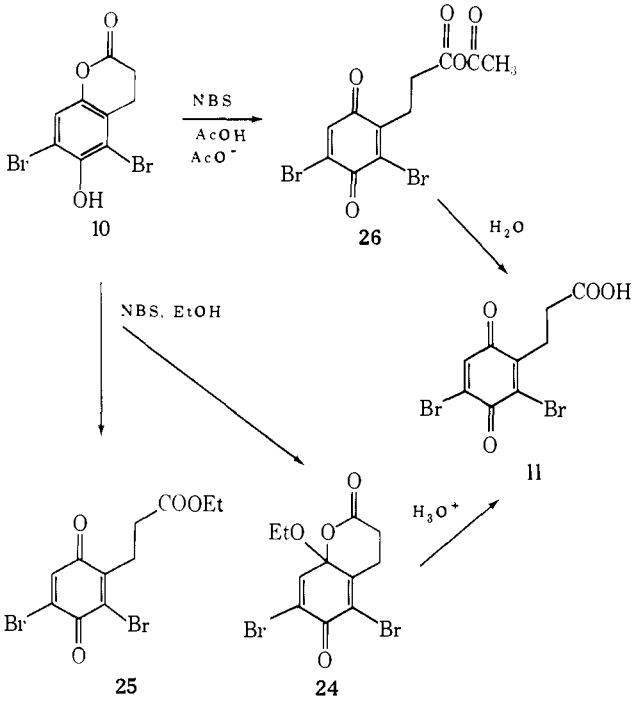
(16) W. John, E. Dietzel, and W. Emte, *Hoppe-Seyler's Z. Physiol. Chem.*, 257, 173 (1939).

(17) The acetoxydienone does not acetylate the aniline under these conditions.

(18) Since *p*-bromoaniline can attack either carbonyl group of the anhydride, only a portion of **14** will transfer the acetyl group; the other product, the *p*-bromoanilide of **6**, is presumed to have been formed but was not isolated.

led, however, only to the mixed anhydride **26** in high yield, and no detectable dienone species (Scheme III).

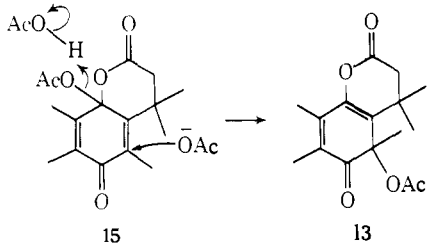
Scheme III



In its selectivity, acetic acid appears to resemble water more than it does ethanol. By analogy with the formation of **25** and carboxyl-labeled **11**, **26** is presumed to arise by acetolysis of **10b**.

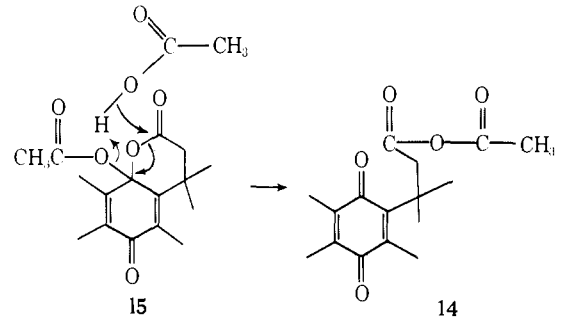
In summary to this point, **5**, **7**, and **10** all form *p*-ethoxydienones in ethanol, but **10** also forms the quinone ester **25**. While **5** forms an *o*-acetoxydienone, **7** forms a *p*-acetoxydienone, and **10** forms no acetoxydienone. Concurrently, **5** and **7** form small quantities of mixed anhydride, while **10** forms only mixed anhydride. Thus, in addition to our need to explain the difference in oxidation behavior between **7** and **10**, the position isomerism found in **13** and **22** presents a further challenge.

Addition of NBS to a solution of **5** in dry acetonitrile produced no spectral change over several hours. Immediately following addition of 2 equiv of tetrabutylammonium acetate,[19] the spectrum changed to that of the para-substituted dienone **15**. In the



presence of a large excess of acetate ion, and at elevated temperature, **15** was transformed into its isomer **13**. In the presence of both acetate ion and acetic acid, the same conversion occurred rapidly at room temperature, the rate increasing with an increase in the concentration of either acetate species (or both). The evidence suggests an SN2′ mechanism for the isomerization,[20] catalyzed by general acid protonation of the leaving group. Of the isomers, **13** is the more stable, no conditions having been found to effect the reverse isomerization to any detectable extent. The thermodynamic preference for **13** may be due to the better accommodation therein for the bond angles of an olefin in the intercyclic position than in the exocyclic position of **15**.[4a] For the same reason, the *o*-acetoxydienone is formed only at C-5 (in contrast to C-7), despite the possibility of steric interference by the 4,4-dimethyl groups. At elevated temperatures, **13** undergoes a further slow isomerization to **16**, presumably *via* a carbonium ion mechanism. The SN2′ reaction is not limited to acetate ion as nucleophile: in hot ethanol, **15** is converted into **18**; this reaction is slower than that with acetate ion and is somewhat less sensitive to acid catalysis;[21] a similar reaction seems to occur with aniline in hot acetonitrile, although the spectrum is partially obscured by by-products. In the presence of acetic acid alone, **15** is not isomerized to **13**, but is transformed into the quinone anhydride **14**. The rate of this conversion increases with the concentration either of **15** or of acetic acid, but catalytic quantities of stronger acids (*e.g.*, trifluoroacetic) seem to have little effect. Assay with *p*-aminocinnamic ester demonstrates a stoichiometry between quinone intensity (uv) and anhydride content.[17]

We suggest that this transformation of **15** to **14** occurs



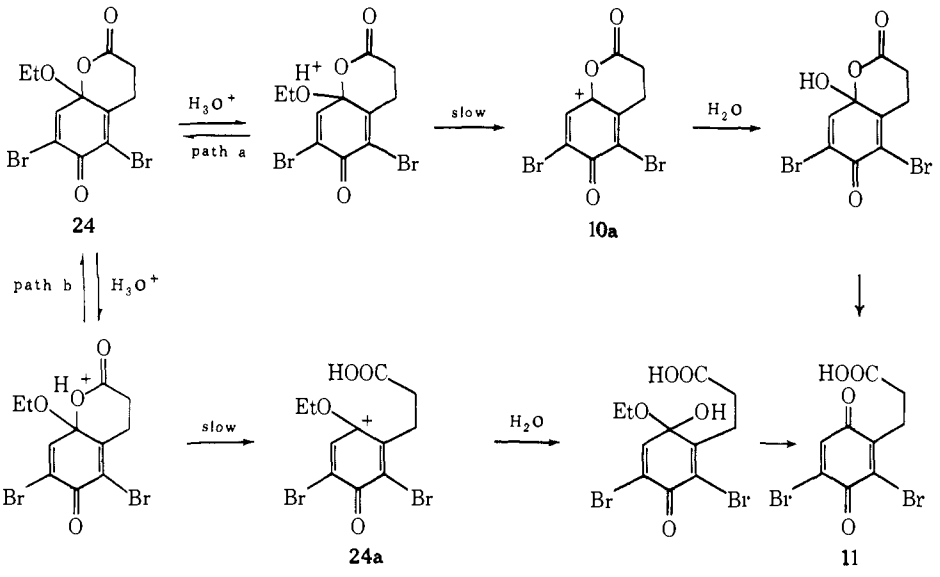
via a cyclic transition state, in which the side-chain carbonyl may acquire some acylium ion character. The ease of this transformation and the almost total conversion of **15** to **14** suggest that the free energy content of **15** must be at least as great as that of **14**. This conversion illustrates an interesting intramolecular energy transfer process, whereby the free energy gained in the resonance stabilization of the alkylquinone system is utilized to drive the synthesis of the high energy anhydride bond.

The sequence of events in the oxidation of **5** in acetic acid–sodium acetate appears to involve (1) formation of **15** as a kinetically determined product, (2) rearrangement of **15** to the more stable product **13** by the combined action of acetic acid and acetate ion, and (3) simultaneous and competitive conversion of **15** into **14** by the action of acetic acid alone. Since **13** and **14** are not interconvertible and undergo no further transformations under the reaction conditions, the ratio of these two products becomes fixed as soon as **15** is depleted. The NBS oxidation of **5** in acetic acid alone proceeds very slowly. This reaction may be repressed by the hydrogen bromide liberated during

(19) One equivalent serves as nucleophile, the other neutralizes the hydrogen bromide released during oxidation.

(20) F. G. Bordwell, *Accounts Chem. Res.*, 3, 281 (1970).

(21) Upon continued heating, **18** rearranges to the ethoxy analog of **16**.



oxidation, or the positive halogen species HBr_3 may be less powerful than NBS;[22] the formation of HBr_3 during the reaction is readily seen by ultraviolet spectroscopy, the intense band at 265 nm disappearing rapidly upon addition of Lewis bases. From the results of oxidation of **5** in the presence of acetate ion, ethanol, or $H_2{}^{18}O$, it is clear that a para-substituted dienone is the initial and, possibly, the exclusive kinetic product.

In contrast to the labilities of **5b** and **15**, the ethoxydienone **12** is inert to further change by any of the reagents operating on **15**, over 24 hr and up to 70°. This difference in reactivity is due, presumably, to the more effective leaving ability of acetate than of alkoxide. In aqueous acid, **12** is hydrolyzed to a mixture of **6** and **17**; a similar conversion occurs with **13**, **14**, **15**, **16**, or **18**. In ethanol containing a trace of ethoxide ion, **12** is converted into the ethyl ester of **6**, undoubtedly by nucleophilic addition to the lactone carbonyl.

The *p*-ethoxydienone obtained from **7** (**21**) shows a stability comparable to that of **12**. The *p*-acetoxydienone **22** is converted into the quinone anhydride **23** by acetic acid, but appears inert to SN2′ rearrangement by acetic acid–acetate ion at 25°. We suggest that resonance stabilization of the vinyl bromide system in **22** is sufficient to outweigh the geometrical preference for the intercyclic double bond of its *o*-acetoxy isomer. In aqueous acid, **21** and **22** are readily transformed into the spiroketone **9**; in the case of **23**, this process occurs simply upon addition of water.

The results with $H_2{}^{18}O$ and with ethanol suggest that a *p*-acetoxydienone is not a requisite initial product in the oxidation of **10** in acetic acid–sodium acetate, and that a mixed anhydride might form directly by acetolysis of **10b**. Indeed, oxidation of **10** in acetonitrile containing 2 equiv of acetate ion produced a quinone spectrum without evidence for a transient dienone. Similar results were obtained with acetic acid–acetate ion; in each case, assay with aminocinnamic ester indicated the yield of anhydride **26** to be at least 90%.

While the *p*-ethoxydienones **12**, **21**, and **24** all hydrolyze in aqueous acid to their respective quinonepropionic acids (at least as initial products), the rate constants ($k_{H_3O^+}$) for these reactions differ significantly. The kinetics of hydrolysis were followed by the decrease in intensity of the dienone chromophore, and were found to obey the (pseudo) first-order rate law over the entire range of acid concentrations studied. Plots of k_{obsd} *vs.* the activity of hydrochloric acid (20% dioxane, 30°, μ = 3.0 *M*) were linear (Figure 1), as required for reactions following eq 1. The intercept

$$k_{obsd} = k_{H_3O^+}(a_{HCl}) + k_{H_2O} \quad (1)$$

values, k_{H_2O}, are very small and may be neglected. For

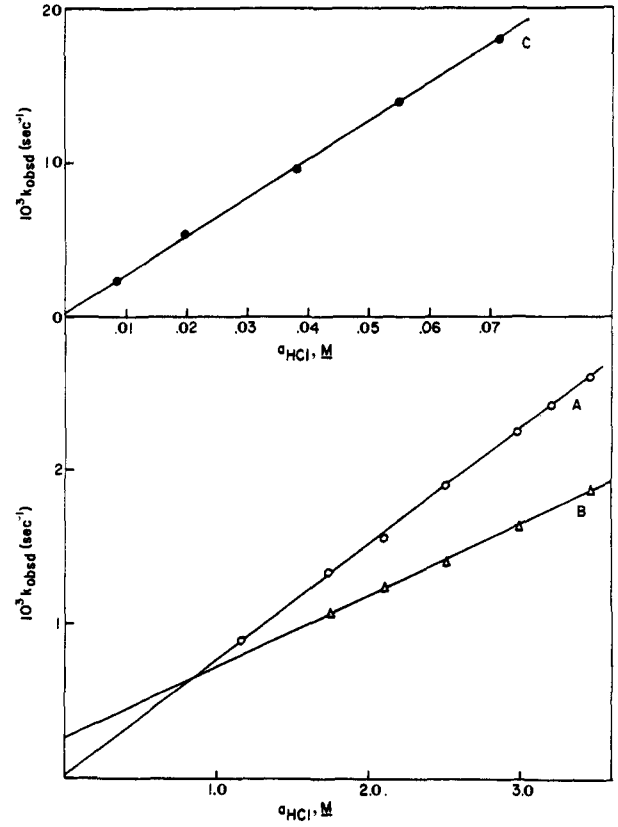
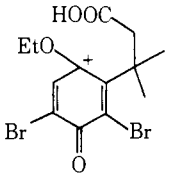


Figure 1. Plots of k_{obsd} for hydrolysis of *p*-ethoxydienones to quinones *vs.* a_{HCl} at 30° in dioxane–water (4:1) (μ = 3.0 *M*): A, **12**; B, **21**, and C, **24**.

(22) G. L. Schmir and L. A. Cohen, *J. Amer. Chem. Soc.*, **83**, 723 (1961).

12, $k_{H_3O^+} = 7.5 \times 10^{-4}\ M^{-1}\ sec^{-1}$; for **21**, $4.7 \times 10^{-5}\ M^{-1}\ sec^{-1}$; and for **24**, 0.25 $M^{-1}\ sec^{-1}$. Thus, **24** is *ca.* 5300 times as sensitive to acid-catalyzed hydrolysis as is its counterpart **21**.

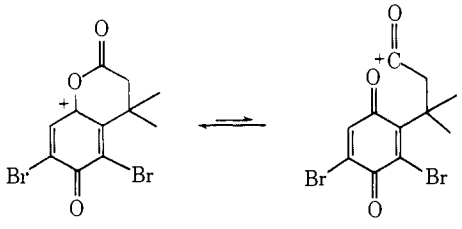
We consider two pathways for these hydrolyses: (1) in path a, rapid, reversible protonation of **24** at the ether oxygen is followed by a slow collapse to the carbonium ion **10a** which, after solvolysis, leads to the quinone **11**; in path b, protonation of the lactone oxygen leads to **11** *via* the carbonium ion **24a**. Of the two sites for protonation, the ether oxygen is undoubtedly the more basic; nevertheless, we favor path b because resonance stabilization of **24a** should be considerably better than that of **10a**.[23] A further argument against path a stems from the fact that **24** is not converted into **25** in ethanolic hydrogen chloride, eliminating consideration of **10b** (or **10a**) as intermediates. The lability of **24**, relative to that of **21** or **12**, is readily understandable on the basis of path b. In **24a**, the carboxyl side chain may unfold and extend sufficiently to permit ready access of water to the carbonium ion. In **21a**



21a

(or in the equivalent species arising from **12**), however, the carboxyl side chain is constrained in a cisoid conformation by the trialkyl lock; there is a much greater probability, now, for intramolecular recapture of the carboxyl group and re-formation of **21** than for intermolecular solvolysis.

The concept of stereopopulation control not only provides the basis for explaining the difference in stability between **21** and **24**, but also clarifies the earlier oxygen-18 results. The NBS oxidation of **10** leads to the carbonium ions **10a** and **10b**. In water and in acetic acid, reaction occurs overwhelmingly with **10b**; in ethanol, reaction occurs with both carbonium ions.[24] The carbonium ions **5a** and **7a** should be favored over



7a **7c**

their acylium ion counterparts (such as **7c**), since the side chains are conformationally constrained to proximity with the ring oxygen atom; consequently, all oxidation products of **5** and of **7** arise *via* **5a** and **7a**, respectively.

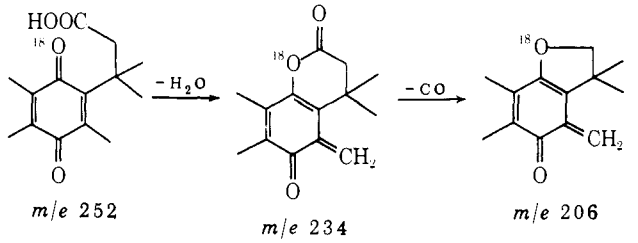
Earlier studies have dealt with the effects of stereopopulation control on rates of cyclization and on ring-chain equilibria.[2a] The present investigation extends the role of the phenomenon to selection between alternative mechanisms and alternative products. Other cases involving divergent mechanisms and products are currently under investigation.

Experimental Section[25]

NBS Oxidation of 5 in $H_2{}^{18}O$. To a stirred solution of **5** (100 mg, 0.43 mmol)[2a] in 4.5 ml of acetonitrile and 0.2 ml of $H_2{}^{18}O$ (90% enriched) was added 80 mg (0.45 mmol) of NBS in 1 ml of acetonitrile. After 2 min, the solvent was removed *in vacuo* (5 min) and the residual material was extracted with benzene. The benzene extract was dried ($MgSO_4$) and evaporated to give a yellow oil. Crystallization from chloroform–hexane gave 72 mg of **6**, mp 99–101.5°; this material was further purified by sublimation, mp 99–102° (lit.[2a] mp 101–103°). Infrared analysis ($CHCl_3$) showed the normal carboxyl band at 1700 cm^{-1} to be intact and the broad quinone band at 1640 cm^{-1} (apparently an unresolved doublet due to the slightly different carbonyls) to have been resolved into two narrow bands at 1640 and at 1615 cm^{-1}, of roughly equal intensity, A displacement of 25 cm^{-1} falls within the range of 20–30 cm^{-1} expected for the isotopic shift.[26]

The mass spectrum of the normal compound shows a parent peak at *m/e* 250 and initial transformations consistent with those of Scheme IV. For the labeled compound, the parent peak appears at *m/e* 252, with a minor signal at *m/e* 250 (10% of the major peak) and none at *m/e* 254. Loss of water produces a signal at *m/e* 234 (minor 232 peak) and further loss of carbon monoxide[27] a signal at *m/e* 206 (minor peak at 204). There appears no significant signal at

Scheme IV



m/e 252 *m/e* 234 *m/e* 206

m/e 20 ($H_2{}^{18}O$) or at *m/e* 30 ($C^{18}O$). These data, in conjunction with the ir analysis, lead to the conclusion that isotopic labeling is present in a quinone carbonyl but not in the carboxyl group.

A sample of normal **6** was stored in the same solvent mixture for 4 hr, and was recovered as above. The ir and mass spectra were indistinguishable from those of **6** which had not been exposed to $H_2{}^{18}O$.

NBS Oxidation of 7 in $H_2{}^{18}O$. From a similar oxidation of **7**, the spirolactone **9** was recovered in 55% yield, mp 126–128°, from acetone–hexane (lit.[2a] mp 125–128°). The ir spectrum of the normal compound ($CHCl_3$) shows the spirolactone carbonyl at 1810 cm^{-1}, the quinone carbonyls at 1700 cm^{-1}, and the olefinic double bond at 1585 cm^{-1}. In the labeled material, the lactone and olefinic bands are unchanged. Two quinone bands of equal intensity appear at 1700 and 1675 cm^{-1}.

The mass spectrum of **9** is more complex than that of **6**, but appears to follow the same fragmentation pattern; as in the earlier case, the spectrum of the labeled compound shows no significant signals at *m/e* 20 or 30. From the mass spectral and ir data, we conclude that labeling has occurred in a quinone but not in the lactone carbonyl. A sample of labeled **9** was stored in 20% aqueous acetonitrile for 8 hr; infrared and mass spectra of the spirolactone, after recovery, showed essentially all of the isotope to have been lost by exchange. In the presence of $H_2{}^{18}O$, unlabeled **9** incorporated almost two atoms of ^{18}O in the same time period.

(23) The poor ability of a lactone oxygen to stabilize the adjacent positive charge corresponds to its inability to overlap with the benzene ring in nitrophenyl esters and lactones; see L. A. Cohen and S. Takahashi, *J. Amer. Chem. Soc.*, **95**, 443 (1973).

(24) This difference in behavior may be interpretable on hard–soft principles, the harder acylium ion **10b** preferring the harder solvents.

(25) All analyses and nmr and mass spectra were provided by the Analytical Services and Instrumentation Section of this laboratory, under the direction of Dr. D. F. Johnson. All melting points are uncorrected.

(26) (a) A. Lapidot, S. Pinchas, and D. Samuel, *Proc. Chem. Soc.*, 109 (1962); (b) S. Pinchas, D. Samuel, and M. Weiss-Broday, *J. Chem. Soc.*, 2382 (1961); (c) E. D. Becker, H. Ziffer, and E. Charney, *Spectrochim. Acta*, **19**, 1871 (1963).

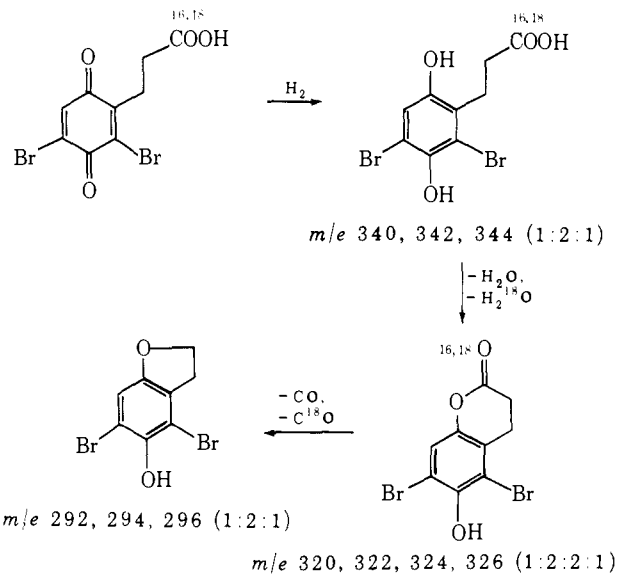
(27) Loss of carbon monoxide from coumarins has been observed previously: R. A. W. Johnstone, B. J. Millard, F. M. Dean, and A. W. Hill, *J. Chem. Soc. C*, 1712 (1966); F. M. Dean, J. Goodchild, R. A. W. Johnstone, and B. J. Millard, *ibid.*, 2232 (1967).

Under comparable conditions, γ-butyrolactone gave no indication of isotope incorporation.

NBS Oxidation of 10 in $H_2{}^{18}O$. From a similar oxidation of **10**, the quinone acid **11** was recovered in 78% yield, mp 169–172°, from acetone–hexane (lit.[2a] mp 172–175°). The ir spectrum of the normal compound (KBr) shows the carboxyl band at 1702 cm^{-1} and the quinone carbonyl bands at 1655 and 1678 cm^{-1}. In the labeled compound, the band at 1702 cm^{-1} has been reduced in intensity while that at 1678 cm^{-1} is more intense and broader. Evidently, the carboxyl band has been displaced (in part) 24 cm^{-1} to overlap with one of the quinone carbonyl bands.

The mass spectral fragmentation pattern of **11** differs slightly from that of **6**. The first step involves reduction of the quinone to the hydroquinone,[28] followed by lactonization and decarbonylation (Scheme V). Although the labeled compound shows isotopic enrichment at the hydroquinone and lactone stages, the decarbonylated material gives a signal similar to that of the unlabeled compound. Furthermore, the labeled compound shows signals at *m/e* 18 (H_2O) and at *m/e* 20 ($H_2{}^{18}O$), as well as peaks at *m/e* 28 (CO) and 30 ($C^{18}O$). The signals at *m/e* 20 and 30 do not appear in the unlabeled compound. After exposure to water for 8 hr, the ir and mass spectra of the labeled compound were unchanged. Accordingly, we conclude the labeling has occurred only in the carboxyl group.

Scheme V



3-(6′-Ethoxy-6′-hydroxy-2′,4′,5′-trimethyl-3′-oxocyclohexa-1′,4′-diene)-3,3-dimethylpropionic Acid Lactone (12). To a stirred solution of **5** (0.40 g, 1.7 mmol) in 25 ml of acetonitrile–ethanol (9:1) was added dropwise, at 25°, a solution of 0.32 g (1.8 mmol) of NBS in 10 ml of acetonitrile. The reaction mixture was stirred for 1 hr, diluted with water, and extracted with several portions of ether. The combined ether fractions were washed with water and saturated brine, and were dried ($MgSO_4$). Removal of the solvent afforded a yellowish oil which was purified by preparative tlc on silica gel (eluent, ethyl acetate–hexane, 3:7). The purified material was crystallized from hexane to give 0.29 g (58%) of **12**: mp 97–99°; ir ($CHCl_3$) 1805 and 1681 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.17 (t, 3 H, CH_2CH_3), 1.38 and 1.45 (2 s, 6 H, C-3 CH_3's),[29] 1.92 and 1.98 (2 s, 6 H, C-4′ and C-5′ CH_3's), 2.13 (s, 3 H, C-2′ CH_3), 2.18 and 2.43 (2 s, 2 H, C-2 CH_2),[29] and 3.15 ppm (q, 2 H, OCH_2CH_3); uv (CH_3OH) λ_{max} 235 nm (ε 13,560) and 279 (2040).

Anal. Calcd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97. Found: C, 69.07; H, 8.04.

Examination of the original reaction mixture by tlc and uv spectroscopy demonstrated the absence of phenolic material, of quinoid material (**6** or its ester), or the *o*-dienone **18**.

3-(2′,4′-Dibromo-6′-ethoxy-6′-hydroxy-3′-oxocyclohexa-1′,4′-diene)-3,3-dimethylpropionic Acid Lactone (21). A procedure similar to that for the oxidation of **5** was used. The yellowish oil obtained after evaporation of ether was purified by chromatography on silica gel (eluent, ethyl acetate–hexane, 1:4). Crystallization of the major fraction (acetone–hexane) afforded 0.76 g (75%) of **21**: mp 132–134°; nmr (acetone-d_6) δ 1.25 (t, 3 H, OCH_2CH_3), 1.56 and 1.68 (2 s, 6 H, C-3 CH_3's),[29] 2.62 and 2.77 (2 s, 2 H, C-2 CH_2),[29] 3.80 (q, 2 H, OCH_2CH_3), and 7.68 ppm (s, 1 H, C-5′ CH_3); uv (CH_3OH) λ_{max} 256 nm (ε 8950).

Anal. Calcd for $C_{13}H_{14}O_4Br_2$: C, 39.62; H, 3.58; Br, 40.56. Found: C, 39.70; H, 3.63; Br, 40.91.

Examination of the original reaction mixture by tlc and uv spectroscopy showed the absence of phenolic material, quinonoid material (ester of **8**), or of spirolactone **9**.

NBS Oxidation of 10 in Ethanol. A procedure similar to that for the oxidation of **5** was used. Following evaporation of solvent, the residual reddish oil was crystallized from chloroform–hexane to give 44% of the quinonepropionic acid **11**, mp 170–173°. The mother liquors were purified by chromatography on silica gel (eluent, ethyl acetate–hexane, 1:4) and provided, after crystallization from hexane, 40% of **25**: mp 57.5–59.5°; uv (CH_3OH) λ_{max} 288 nm (ε 9900).

Anal. Calcd for $C_{11}H_{10}O_4Br_2$: C, 36.09; H, 2.75; Br, 43.67. Found: C, 36.12; H, 2.80; Br, 43.49.

The uv spectrum of the reaction mixture, immediately following addition of NBS, showed a peak at 288–290 nm (**25**) and a shoulder at 260–263 nm (**24**). Slow hydrolysis of **24** resulted in a decrease in absorbance at 260 nm and a concomitant increase at 290 nm. The breakdown of **24** could be retarded significantly by addition of tetrabutylammonium acetate to the cuvette after oxidation had been completed, the salt serving to neutralize the hydrogen bromide formed during oxidation. Despite the same precautions on a preparative scale, every attempt to isolate **24** by preparative tlc on silica gel, cellulose, alumina, or Florisil failed. The yield of **25** was not altered significantly by a 24 hr delay in work-up of the reaction mixture, or by addition of acetate ion or acetic acid prior to work-up. Addition of 3% ethanolic hydrogen chloride to the original mixture of oxidation products resulted in rapid destruction of **24**; however, the yield of **25** was not increased, the dienone being recovered as **11** in the work-up.

Oxidation of 5 in Acetic Acid. A. With Aqueous Work-up. To a stirred solution of **5** (0.94 g, 4.0 mmol) and 2.20 g of fused sodium acetate in 40 ml of anhydrous acetic acid[30] was added dropwise, at 45°, a solution of NBS (0.71 g, 4.0 mmol) in 20 ml of acetic acid. The yellowish solution was stirred at 25° for 30 min and was lyophilized. To the residue was added 15 ml of water and the mixture was extracted with several portions of ether. The combined ether fractions were washed with water, 5% sodium bicarbonate, water, and saturated brine, and were dried ($MgSO_4$). Removal of solvent and crystallization of the residue from ether–hexane afforded 0.91 g (78%) of the *o*-acetoxydienone **13**: mp 108–111°; ir ($CHCl_3$) 1770 (lactone C=O), 1740 (acetate C=O), and 1665 cm^{-1} (ketone C=O); nmr ($CDCl_3$) δ 1.12 and 1.32 (2 s, 6 H, C-3 CH_3's),[29] 1.48 (s, 3 H, C-2′ CH_3), 1.88 and 2.02 (2 s, 6 H, C-4′ and C-5′ CH_3's), 2.03 (s, 3 H, $COCH_3$), and 2.41 ppm (s, 2 H, C-2 CH_2); uv (CH_3OH) λ_{max} 327 nm (ε 3000).

Anal. Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 66.01; H, 6.71.

The sodium bicarbonate extract was acidified with 5% hydrochloric acid and was extracted with several portions of ether. The combined ether extracts were washed with water and saturated brine and were dried ($MgSO_4$). Removal of the solvent and crystallization from acetone–hexane gave 0.16 g (16%) of **6**, mp 98–101°.

B. With *p*-Bromoaniline Work-up. The oxidation of **5** was repeated as above; prior to lyophilization, a solution of purified *p*-bromoaniline (0.69 g, 4.0 mmol) in 20 ml of benzene was added and the mixture was stirred at 25° for 1 hr. The residue obtained after lyophilization was dissolved in 75 ml of ethyl acetate, the solution was filtered, and the filtrate was washed with water, 5% sodium bicarbonate, 5% hydrochloric acid, water, and saturated brine, and was dried ($MgSO_4$). Removal of solvent gave a reddish oil which was purified by chromatography on silica gel (eluent, ethyl acetate–hexane, 3:7). The faster moving fraction was crystallized from ether–hexane to give 0.90 g (76%) of **13**, mp 107–

(28) Reduction to the hydroquinone prior to fragmentation is a feature common to a number of quinones: H. A. Lloyd, E. A. Sokolski, B. S. Strauch, and H. M. Fales, *Chem. Commun.*, 299 (1969); R. F. Muraca, J. S. Whitlock, G. D. Daves, Jr., P. Friis, and K. Folkers, *J. Amer. Chem. Soc.*, **89**, 1505 (1967); P. J. Rietz, F. S. Skelton, and K. Folkers, *Int. Z. Vitaminforsch.*, **37**, 405 (1967); B. C. Das, M. Lounasmaa, C. Tendrille, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **21**, 318 (1965); H. Morimoto, T. Shima, I. Imada, M. Sasaki, and A. Ouchida, *Justus Liebigs Ann. Chem.*, 702, 137 (1967).

(29) The magnetic nonequivalence at these centers is due to the asymmetric substitution at C-6′.

(30) The solvent was purified by distillation from potassium permanganate and dried by distillation from anhydrous copper sulfate.

110°. The slower moving fraction was crystallized from ethyl acetate–hexane to give 0.06 g (7%) of *p*-bromoacetanilide, mp 166.5–168°. The sodium bicarbonate extract was acidified as described above, to give 0.06 g (6%) of the quinonepropionic acid **6**, mp 98–102°.

Oxidation of 7 in Acetic Acid. Oxidation with NBS was performed as described above for **5**. Following work-up, the residual reddish oil was purified by chromatography on silica gel (eluent, ethyl acetate–hexane, 1:1). The faster running material was crystallized from acetone–hexane to give 0.064 g (15%) of *p*-bromoacetanilide.[31] The slower running fraction was crystallized from ether–hexane to give 0.48 g (61%) of the *p*-acetoxydienone **22**: mp 140–143°; ir ($CHCl_3$) 1788, 1765, and 1691 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.62 and 1.65 (2 s, 6 H, C-3 CH_3's), 2.13 (s, 3 H, $COCH_3$), 2.53 (d, 1 H, J = 15 Hz, C-2 CH), 3.15 (d, 1 H, J = 15 Hz, C-2 CH), and 7.46 ppm (s, 1 H, C-5′ CH); uv (CH_3OH) λ_{max} 259 nm (ϵ 5900).

Anal. Calcd for $C_{13}H_{12}O_3Br_2$: C, 38.26; H, 2.96. Found: C, 38.42; H, 3.15.

In a second run with aqueous work-up, silica gel chromatography provided 68% of **22** and 24% of the spirolactone **9**, mp 126–128° (acetone–hexane).

Oxidation of 10 in Acetic Acid. The procedure described by Thanassi and Cohen[5] was repeated with the same results with respect to yields and nature of products. A careful search for acetoxydienones, both by uv spectroscopy and tlc, was fruitless. Aqueous work-up led to recovery of 85% of **11**, again without detection of acetoxydienones. Aminocinnamic ester assay indicated a minimum of 90% of **26** in the oxidation mixture.

Formation of 15 and Its Transformations. To a solution of **5** in dry acetonitrile (5×10^{-5} *M*) was added an equivalent amount of NBS. The spectrum (uv) remained unchanged over 4 hr. Addition of 2 equiv of tetrabutylammonium acetate effected rapid loss of the phenolic chromophore and generation of a new band at 237–238 nm, which reached a maximum intensity within 3 min. This spectrum remained unchanged for at least 2 hr; further addition of 10–100 equiv of acetate ion effected a low shift of this band to a new chromophore at 325–330 nm. The rate of this transformation increased with the amount of acetate ion added, as well as with temperature. The new product (λ_{max} 325–330 nm) was identified as **13** by tlc and mass spectrum (isolated from a larger scale run). Several attempts to isolate the intermediate *p*-acetoxydienone **15** resulted in its decomposition to a mixture of **13** and **6**. Addition of acetic acid to the reaction mixture increased the rate of conversion of **15** to **13** significantly. When the mixture of **15** and acetate ion was maintained at 60–70° overnight, the spectrum changed once again to a low intensity peak at 288–290 nm. This peak shifted to 300 nm upon addition of tetrabutylammonium methoxide and reverted to its original position upon acidification of the mixture; the spectrum is considered to characterize a phenolic system, presumably that of **16**. The same rearrangement was effected with **13** alone at elevated temperatures (without any added acetate ion).

Addition of 10% ethanol to the solution of **15** in acetonitrile produced no spectral change at 25°; at 60–70°, however, the *p*-dienone peak was lost and a new chromophore appeared at 325–330 nm. On silica gel plates, this material ran slightly slower than **13**, and it is presumed to be **18**, the ethoxy analog of **13**. As in the case of **13**, prolonged heating of the solution of **18** effected isomerization to a phenolic system, the ethoxy analog of **16**.

Addition of glacial acetic acid to the solution of **15** in acetonitrile effected a shift in spectrum from that of a para-substituted dienone (235–240 nm) to that of the quinone chromophore (258–260 nm). The rate of this conversion increased with acetic acid concentration; in the presence of 10% acetic acid, the conversion appeared to be complete in 20–30 min at 25°. Since the quinone chromophore was not detected in an acetic acid–acetate ion mixture, the conversion of **15** to **13** is evidently faster than that of **15** to **14**. No significant spectral change was observed when a small amount of trifluoroacetic acid was substituted for acetic acid; consequently, the acetyl moiety in the mixed anhydride function of **14** is presumed to come from the added acetic acid, and not *via* an intramolecular rearrangement. Assay of the solution of **14** with *p*-aminocinnamic ester showed the product to be essentially all anhydride, based on calculations which assume the ϵ value of **14** to be identical with that of **6**.

Kinetic Measurements. Rates of hydrolysis of the *p*-ethoxydienones **12**, **21**, and **24** were obtained by adding aliquots of stock solutions in dioxane to dioxane–water (4:1) containing varying amounts of hydrochloric acid. The rate of decrease of uv intensity was recorded with time at 235 nm in the case of **12**, and at 256 nm in the cases of **21** and **24**. Since **24** could not be isolated, aliquots of a stock solution in 10% ethanolic acetonitrile were used. Equipment and techniques were the same as those previously described.[2a] All kinetic runs were made at 30 ± 0.05°, in duplicate or triplicate, and followed the first-order rate law with least-squares correlation coefficients of 0.999 or better. The activity values of hydrochloric acid in aqueous dioxane were obtained from the data of Harned and Owen.[32] Ionic strength was maintained at 3.0 *M* with potassium chloride; the effect of the salt on a_{HCl} was neglected.

Spectrophotometric Assay for Carboxylic Anhydrides. This assay method is based on the decrease in uv absorption produced by acylation of anilino nitrogen. Since some of the compounds under investigation in this study themselves have absorption at wavelengths as long as 330 nm, simple anilines of adequate nucleophilicity would have their spectra totally obscured. Ethyl *p*-aminocinnamate, with λ_{max} at 332 nm, and with significant absorption even at 380 nm, was found effective in overcoming these obstacles. The anilino group is sufficiently basic to be acetylated by acetic anhydride, moderately fast and with sufficient accuracy for most requirements at 25–50°.

A stock solution of the reagent (2.25×10^{-3} *M*) was prepared in dry acetonitrile (distilled twice from phosphorus pentoxide). Addition of 0.1 ml of this solution of 2.5 ml of dry acetic acid (or any other solvent unreactive toward acid anhydrides) gave OD = 1.22 at 332 nm and 0.57 at 360 nm. In acetic acid, acetic acid–sodium acetate, or in the presence of any of the acetoxydienones described herein, the spectrum was unchanged after 4 hr at 25° and an additional 2 hr at 50°. Addition of 2 equiv of acetic anhydride reduced the OD at 260 nm to 0.10 in 2 hr at 25° or 20 min at 50°. An aliquot of the reaction mixture was taken such that the cuvette contained a twofold excess of reagent over the maximum possible content of acid anhydride. After the initial spectrum had been recorded, the cuvette was stoppered tightly and stored in a 50° oven. Spectra were recorded at intervals until no further decrease in absorbance at 360 nm was evident (usually 1–2 hr). The anhydride content of the sample was then obtained as $\Delta OD_{obsd}/\Delta OD_{calcd}$.

(31) No attempt was made to recover the small amount of **9** undoubtedly present.

(32) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold, New York, N. Y., 1958, p 717.