Lactonization of *cis,cis*-3-Halomuconates: Influence of pH and Halo Substituent on the Regiochemistry

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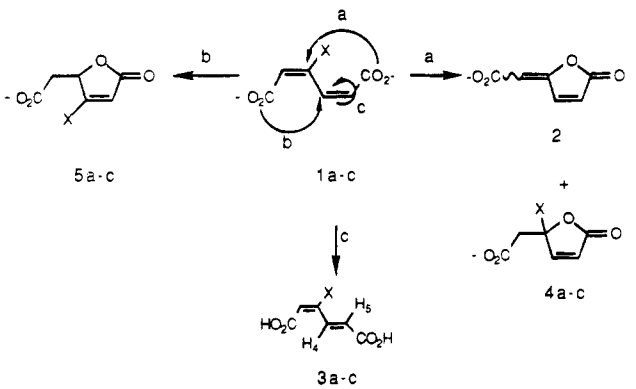
The (2*E*,4*Z*)-3-fluoro-2,4-hexadienedioate (**1a**; *cis,cis*-3-fluoromuconate) and its chloro **1b** and bromo **1c** analogues, which are important metabolites in the microbial degradation of halogenated aromatic pollutants, lactonize under acidic conditions. Two modes of lactonization can occur involving either carboxylate group. We show here that the lactonization and stereomutation of **1a–c** are dependent not only on the pH but also on the halo substituent. The major product from reaction of **1a–c** at pH 1–6, (2,5-dihydro-5-oxofuranylidene)acetic acid (**2**), arose from attack of the C-6 carboxylate on the halide-bearing carbon and subsequent expulsion of the halide. The rate of formation of **2** was maximal at pH 3–4 and approached zero at pH 0 and 7. At pH 3.2 and below, reaction of **1b** and **1c**, but not **1a**, produced the (2*E*,4*E*)-3-halo-2,4-hexadienedioates as additional products. At pH 0 the major product from reaction of **1a**, 2,5-dihydro-2-fluoro-5-oxofuranacetic acid (**4a**), was due to lactonization via the C-6 carboxylate. The 3-chloro- and -bromomuconates (**1b,c**), in contrast, lactonized at pH 0 via attack of the C-1 carboxylate on the unsubstituted C-4 to the 2,5-dihydro-3-halo-5-oxofuranacetic acids. The mechanism of the observed pH and substituent-dependent changes in regiochemistry of lactonization is discussed.

Halogenated aromatic compounds comprise a major class of environmental pollutants. The microbial degradation of these substances is thus the focus of much research activity.[2] In a crucial step of the detoxification process, the halide substituent must be eliminated from the carbon skeleton. *Pseudomonas sp. B13* has been reported to achieve this conversion, as part of a modified β-ketoadipate pathway, through the action of a muconate cycloisomerase on the intermediate *cis,cis*-3-halomuconates **1a-c**.[3] These metabolites are chemically unstable in acidic media, undergoing cycloisomerization as well as C-4,C-5 double-bond isomerization (Scheme I).[4,5] This instability has caused some uncertainty in the literature over the chemical versus enzymatic origin of isolated metabolites[4] and over their configuration.[6,7]

The instability of the *cis,cis*-3-halomuconates can be attributed to the steric and stereoelectronic repulsion that the 3-halide substituent and the C-6 carboxyl group exert on each other. In an anologous case it has been shown that the 6-methyl ester group of dimethyl (2*Z*,4*Z*)-3-methyl-2,4-hexadienedioate is rotated out of the plane of the diene system by 40°.[8] In principle, *cis,cis*-muconates **1a–c** have three different modes of isomerization available to relieve this strain: lactonization involving attack of the C-6 carboxyl group on C-3 to yield diene lactone **2** and/or 4-halomuconolactones **4a–c** (path a, Scheme I); lactonization via attack of the C-1 carboxyl group on C-4 to afford 3-halomuconolactones **5a–c** (path b, Scheme I); or C-4,C-5 double-bond isomerization resulting in the formation of *cis,cis*-3-halomuconates **3a–c**, which are biologically inactive (path c, Scheme I).[4]

Our interest in the mechanism and stereochemistry of muconate cycloisomerases from various organisms has prompted us to investigate the conditions of the nonenzymatic isomerization of *cis,cis*-3-halomuconates. These efforts have been facilitated by our recently reported chemical syntheses of these compounds.[9] We report here the results of this study and propose mechanisms consistent with our findings.

Scheme I



a: X= F, b: X= Cl, c: X= Br

Results

Reaction of *cis,cis*-3-Halomuconates at pH 1–6. Schmidt and Knackmuss observed the cycloisomerization of enzymatically prepared solutions of *cis,cis*-3-halomuconates **1a** at pH 4 and **1b** at pH 5, respectively (100 mM Tris-HCl), to (5-oxo-2,5-dihydrofuran-2-ylidene)acetic acid (**2**, diene lactone).[3] They reported a 9/1 product mixture of (*E*)- and (*Z*)-diene lactones **2a** and **2b** from the reaction of 3-chloromuconate (**1b**) on the basis of HPLC analysis. In addition, they noted that *cis,trans*-3-chloromuconic acid (**3b**) can be formed by exposure of the *cis,cis*-muconate **1b** to pH <1. In order to gain a better understanding of this apparent change in reaction mode, we have repeated these reactions with synthetic *cis,cis*-3-halomuconates **1a–c** over an extended pH range. We monitored the decomposition of **1a–c** in acidic media between pH 1 and 6 by ^{1}H NMR and by UV. A detailed analysis of these data revealed that the exact composition of the product mixture depended not only on the pH of

(1) American Cancer Society Faculty Research Awardee 1983–1988.
(2) Mller, R.; Lingens, F. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 779.
(3) Schmidt, E.; Knackmuss, H.-J. *Biochem. J.* **1980**, *192*, 339.
(4) Schmidt, E.; Remberg, G.; Knackmuss, H.-J. *Biochem. J.* **1980**, *192*, 331.
(5) Evans, W. C.; Smith, B. S. W.; Moss, P.; Fernley, H. N. *Biochem. J.* **1971**, *122*, 509.
(6) Schreiber, A.; Hellwig, M.; Dorn, E.; Reinecke, W.; Knackmuss, H.-J. *Appl. Env. Microbiol.* **1980**, *39*, 58.
(7) Tiedje, J. M.; Duxbury, J. M.; Alexander, M.; Dawson, J. E. *J. Agric. Food Chem.* **1969**, *17*, 1021.
(8) Jarozewski, J. W.; Ettling, M. G. *J. Org. Chem.* **1982**, *47*, 1212.
(9) Pieken, W. A.; Kozarich, J. W. *J. Org. Chem.* **1989**, *54*, 510.

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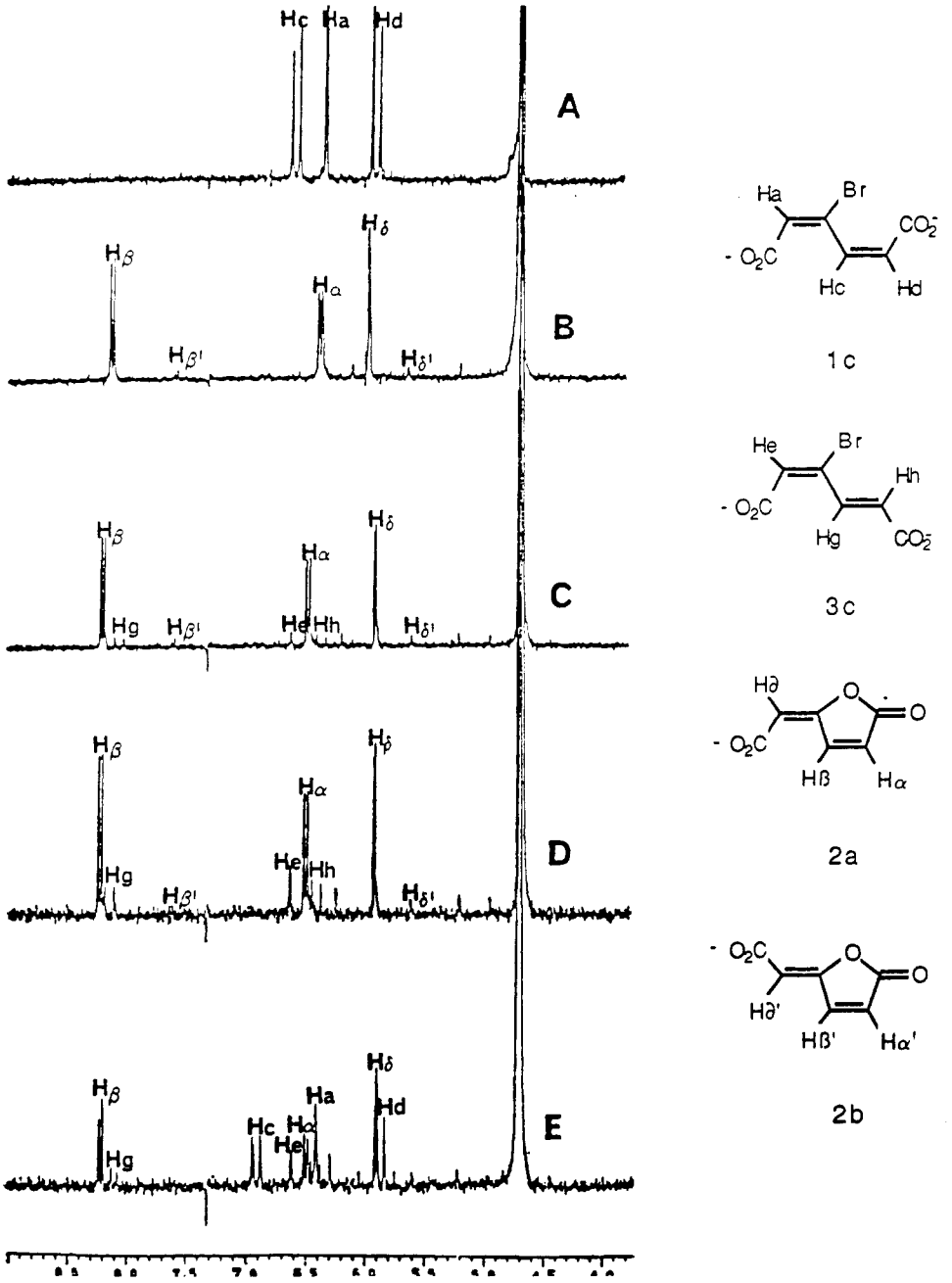


Figure 1. Product mixtures from cycloisomerization of 3-bromomuconate (**1c**) independent of pH. The ^{1}H NMR spectra (pH buffer, 200 MHz) were obtained after 1 h of exposure of 3-bromomuconate (**1c**) to the pH. Key: A, **1c** after 1 h at pH 8.0, no change in the spectrum; B, **1c** after 1 h at pH 5.4; C, **1c** after 1 h at pH 3.2; D, **1c** after 1 h at pH 2.5; E, **1c** after 1 h at pH 1.1.

the reaction medium but also on the nature of the halide substituent.

For the ^{1}H NMR experiments a solution of the halomuconate in aqueous deuteriated buffer (100 mM KD_2PO_4/D_2O, 1 mL), which was previously adjusted with either dilute DCl or NaOD to the desired pH (8.0, 5.4, 3.2, 2.5, 1.1), was allowed to react for 1 h at 25 °C. At the time the reaction solution was analyzed by ^{1}H NMR (400 MHz). The spectra of the reaction of *cis,cis*-3-bromomuconate are shown in Figure 1. The reaction at pH 8.0, which served as a reference showed only the resonances of the initial *cis,cis*-3-bromomuconate (**1c**): δ 6.65 (dd, J = 12.6 Hz, J = 1 Hz), 6.38 (d, J = 1 Hz), 5.95 (dd, J = 12.6 Hz, J = 0.7 Hz). The spectrum of the solution at pH 5.4 showed complete conversion of the initial muconate to a 16/1 mixture of (*E*)-diene lactone **2a** and (*Z*)-diene lactone **2b**, respectively. **2a**: δ 8.15 (d, J = 5.7 Hz), 6.40 (d, J = 5.7 Hz), 5.99 (s); **2b** δ 7.57 (d, J = 5.7 Hz), 6.39 (d, J = 5.7 Hz), 5.61 (s). The ^{1}H NMR signals of **2a** and **2b** agreed with the values reported in the literature.[5] In addition to these resonances, at pH 3.2 a small amount (4%) of *cis,trans*-3-bromomuconate (**3c**) appeared. **3c**: δ 8.16 (d, J = 15 Hz), 6.64 (s), 6.42 (d, J = 15 Hz). As the pH was lowered, the relative amount of the cis,trans isomer **3c** increased to 16% at pH 1.1 (Table I). At pH 1.1 a substantial amount of

Table I. Relative Yields[a] (%) of Reaction Products from Cycloisomerization of 3-Bromomuconate (1c) at Different pH

pH	2a	2b	3c	1c
5.4	94	6	*b*	*b*
3.2	88	8	4	*b*
2.5	77	8	15	*b*
1.1	41	3	16	40

[a] Determined by integration of ^{1}H NMR spectra (Figure 1). [b] Not detected by ^{1}H NMR.

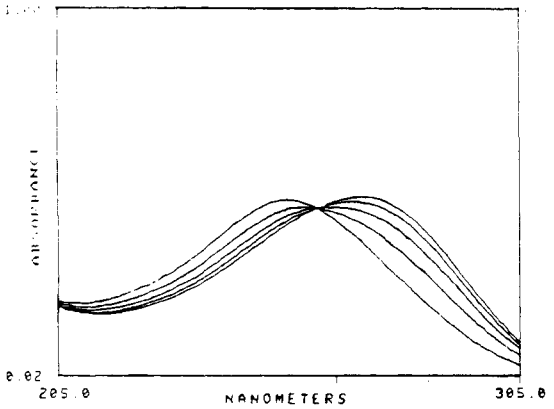


Figure 2. UV time course of the cycloisomerization of *cis,cis*-3-fluoromuconate at pH 1.6. The reaction mixture (50 mM KH_2PO_4, 1-mL volume, 37 μM 3-fluoromuconate (**1a**)) was adjusted to pH 1.6 with dilute HCl. The absorbance between 305 and 205 nm was measured with 0.2-min delay between spectra.

the initial cis,cis acid **1c** (40%) remained unconverted after 1 h (Figure 1, Table I). The results for the reaction of *cis,cis*-3-chloromuconate (1b) were analogous.

The behavior of *cis,cis*-3-fluoromuconate (**1a**) deviated significantly from the chloride and bromide analogues at ≤pH 3.2. Over the observed pH range (pH 1–6), no formation of the cis,trans acid **3a** was detected in the ^1H NMR spectrum. The only observed products were the diene lactones **2**. Furthermore, after 1-h reaction at pH 1.1, a smaller amount of *cis,cis*-acid **1a** (13%) remained unconverted than seen with the heavier halide analogues. This difference in the distribution of reaction products between fluoromuconate and the chloride and bromide analogues was more pronounced at even lower pH, as discussed below.

Since the above reactions of the *cis,cis*-3-halomuconates between pH 1 and 6 were carried out in deuteriated solutions, we also probed for possible deuterium incorporation into the products. Evaluation of the peak integrations of the ^1H NMR spectra revealed that no deuterium was incorporated into any of the products **2a**, **2b**, or **3b,c** (Figure 1). This aspect had not been investigated previously for the acid-induced decomposition of 3-halomuconates. Kirby et al.,[10] however, have demonstrated that the acid-induced cis,cis to cis,trans isomerization of 3-carboxymuconic acid also proceeds without inclusion of deuterium.

The above ^1H NMR study of the *cis,cis*-3-halomuconates between pH 1 and 6 indicated that conversion of the *cis,cis*-muconates is significantly faster between pH 6 and 2 than at pH 8 and pH 1 (Table I). The major product, (*E*)-diene lactone **2a** arises from lactonization. Schmidt and Knackmuss also observed that muconates **1a** and **1b** cycloisomerize faster at pH 4 and 5, respectively, than at pH 6.[3] We thus further probed the pH dependence of the rate of lactonization by monitoring the formation of diene lactone **2** by UV. A typical UV time course of the cycloisomerization of the *cis,cis*-3-halomuconates is shown in Figure 2 for the reaction of *cis,cis*-3-fluoromuconate in aqueous buffer (50 mM KH_2PO_4, pH 1.6). The initial absorbance at 258 nm decreased over time concomitant with an increase in absorbance at 277 nm, with an isosbestic point at 263 nm. The pH–rate profile (Figure 3) of the lactonization of **1a–c** in acidic aqueous media (50 mM KH_2PO_4, pH 6.5, 5.1, 4.1, 3.6, 3.0, 1.6; 1 N H_2SO_4, pH 0.25) was obtained by measuring the initial increase in absorbance at 277 nm. The formation of the cis,trans acids **3b,c**

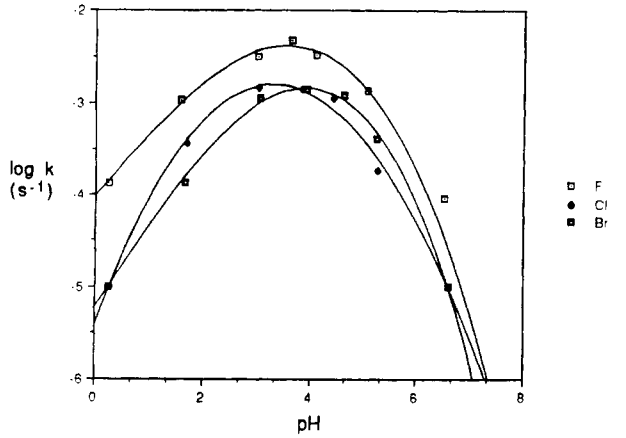


Figure 3. pH–rate profile of diene lactone **2** formation from 3-halomuconates **1a–c**. Reaction mixtures (50 mM KH_2PO_4, 1-mL volume, 37 μM 3-halomuconate concentration) were adjusted to the desired pH values (pH 6.5, 5.1, 4.1, 3.6, 3.0, 1.6) with dilute HCl or NaOH. The pH 0.25 buffer consisted of 1 N H_2SO_4. The increase in absorbance at 277 nm was monitored over 3 min.

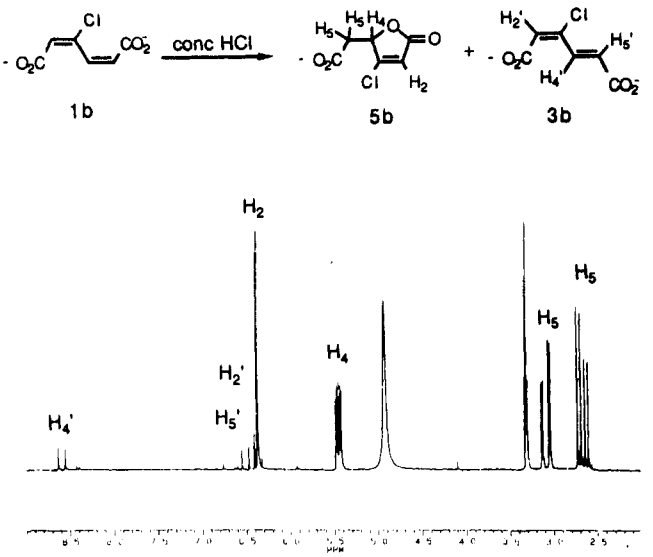


Figure 4. ^1H NMR analysis of the product mixture from the reaction of *cis,cis*-3-chloromuconate (**1b**) in concentrated HCl. The residue from evaporation of the reaction mixture of **1b** in concentrated HCl was taken up in 1 mL of methanol-d_4. The spectrum was obtained at 400 MHz. Chemical shifts are referenced to the resonance of methanol at 3.30 ppm.

did not interfere in these experiments since their UV absorbance only marginally differs from that of the initial *cis,cis* acids **1b,c**. The results in Figure 3 clearly show a maximal rate of diene lactone information between pH 3 and 4. The rate approached zero at pH 7 and 0. It is also apparent that the maximal rate of cyclisomerization of 3-fluoromuconate was higher than that of its chloride and bromide analogues.

Reaction of *cis,cis*-3-Halomuconates in Concentrated HCl. There is only fragmentary evidence in the literature for the acid-induced reaction of *cis,cis*-3-halomuconates below pH 1. As mentioned above, it has been noted by earlier workers that 3-chloromuconate (**1b**) isomerizes to its cis,trans isomer **3b** at pH <1.[4,5] There is also indirect evidence in the early literature for the lactonization of *cis,cis*-3-chloromuconate to the 3-chloromuconolactone via attack of the C-1 carboxylate on C-4 in strong acidic media (path b, Scheme I).[11] The oxidation

(10) Ainsworth, A. T.; Kirby, G. W. *J. Chem. Soc. (C)* **1968**, 1483.

(11) Neunhofer, O. *Ber. Dtsch. Chem. Ges.* **1935**, *68*, 1778.

Table II. Relative Product Distribution[a] (%) from the Reaction of *cis,cis*-3-Fluoromuconate (1a) in Concentrated Acid

reaction medium	2a	2b	4a
HCl	21	2	77
DCl	40	11	49
H_2SO_4	56	44	*b*
D_2SO_4	58	42	*b*

[a] Determined by integration of ^1H NMR spectra. [b] Not detected by ^1H NMR.

of 4-chloro-2-nitrophenol in concentrated H_2SO_4 was observed to produce β-chloromuconolactone (**5b**), presumably via the intermediate *cis,cis*-3-chloromuconic acid. We could thus expect to obtain a mixture of **3b** and **5b** from the reaction of **1b** at pH 0.

We investigated the isomerization of *cis,cis*-3-chloro **1b** and *cis,cis*-3-bromomuconate **1c** in concentrated HCl at room temperature. After complete reaction (1 h) the mixtures were evaporated to dryness and analyzed by ^1H NMR. The product mixture from the conversion of 3-chloromuconate (**1b**) (Figure 4) was composed of 92% 3-chloromuconolactone (**5b**) (δ 6.36 (d, J = 1.8 Hz), 5.44 (m), 3.08 (dd, J = 16.8 Hz, J = 3.5 Hz), 2.66 (dd, J = 16.8 Hz, J = 7.9 Hz)) and 8% *cis,trans*-3-chloromuconic acid (**3b**) (δ 8.59 (dd, J = 15.4 Hz, J = 0.6 Hz), 6.49 (dd, J = 15.4 Hz, J = 0.6 Hz), 6.39 (t, J = 0.6 Hz)). 3-Bromomuconate (**1c**) formed 86% 3-bromomuconolactone (**5c**) and 14% *cis,trans*-3-bromomuconic acid (**3c**) under the same conditions. When concentrated DCl was employed as the reaction medium, the only incorporation of deuterium was found at the C-5 position of 3-halomuconolactones **5b,c**.

To our surprise, the reaction of *cis,cis*-3-fluoromuconate (**1a**) in concentrated HCl at room temperature gave a product mixture markedly different from that seen with the heavier halide analogues. ^1H NMR analysis of the solid residue left after evaporation of the reaction solution showed the formation of 21% (*E*)-diene lactone **2a** accompanied by 2% (*Z*)-diene lactone **2b** and 77% 4-fluoromuconolactone (**4a**) (Table II). The spectral data of **4a** agree with values given in the literature.[12] No resonances corresponding to 3-fluoromuconolactone (**5a**) or *cis,trans*-3-fluoromuconic acid (**3a**) were detected. Thus, *cis,cis*-3-fluoromuconate (**1a**) lactonized with regiochemistry opposite to that of the chloride and bromide analogues under these conditions. It is conceivable that **2** and **4a** arise from a common reaction pathway as depicted in path a, Scheme I, by partitioning of a common intermediate. Although it is not a necessary condition, a product deuterium isotope effect would imply the existence of such an intermediate. When the reaction was carried out in concentrated DCl, the product ratio shifted in favor of the diene lactone to 51%/49% **2**/**4a** (Table II). This corresponds to a product deuterium isotope effect of 3.5.[13] Lactone **4a** was monodeuteriated at the C-5 position; no other deuterium incorporation was detectable.

Reaction of *cis,cis*-3-Fluoromuconate in Concentrated H_2SO_4. In order to test the possibility that the regiospecificity of lactonization might change at even higher acid strength to that observed for the heavier halide substrates **1b,c**, *cis,cis*-3-fluoromuconate (**1a**) was reacted in concentrated H_2SO_4 at room temperature for 1 h. In this medium the product mixture isolated after dilution of the reaction solution with water and extraction with ether revealed a 56%/44% ratio of diene lactones **2a** and **2b** (Figure 5, Table II). No 4-fluoromuconolactone was observed under these conditions. When concentrated D_2SO_4 was employed as the reaction medium under the same conditions, the product ratio of diene lactones **2a** and **2b** remained essentially the same (58%/42%, Table II). In contrast to the reaction in DCl the diene lactones formed in D_2SO_4 showed 50% incorporation of deuterium into the C-5 position (Figure 5).

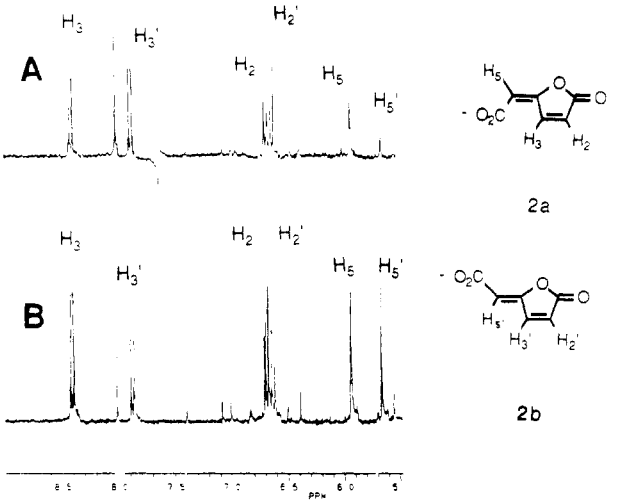


Figure 5. ^1H NMR analysis of the product mixture from the reaction of *cis,cis*-3-fluoromuconate (**1a**) in concentrated sulfuric acid. The residue from evaporation of the ether extract of the reaction of **1a** in concentrated H_2SO_4 and concentrated D_2SO_4 was taken up in 1 mL of D_2O. The spectra were obtained at 200 MHz. Chemical shifts are referenced to the resonance of methanol at 4.70 ppm. Key: A, product mixture from reaction of **1a** in D_2SO_4; B, product mixture from reaction of **1a** in H_2SO_4.

To examine the role of 4-halomuconolactones as intermediates in diene lactone formation at moderate acidic conditions (pH 1–6), we probed the stability of **4a** at pH 7.0 (100 mM KD_2PO_4, D_2O) by ^1H NMR. No change in the spectrum of **4a** had occurred after 4 h.

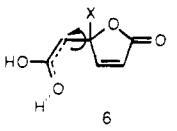
Discussion

The results outlined above establish the pronounced dependency of the lactonization and stereomutation of *cis,cis*-3-halomuconates **1a–c** on the pH of the reaction medium and the nature of the halide substituent. The observed modes of reaction and isolated products are consistent with the notion that acid-induced reaction of these compounds occurs via attack of a carboxylate on an electrophilic carbon center (Scheme I), as discussed in detail below. The crucial role of the electrophilicity of the attacked carbon becomes apparent in a comparison of the reactivity of the *cis,cis*-3-halomuconates to the parent unsubstituted muconic acid. The latter is stable to acidic media, even at 10% HCl.[13] Lactonization of *cis,cis*-muconic acid requires prolonged reaction in 75% sulfuric acid[14] and does not proceed in concentrated HCl even after 24-h reaction time (data not shown). Our data suggest that the mode of activation of the electrophilic reaction center directs the regiochemistry of the lactone formation.

Reaction at pH 1–6. The bell-shaped pH–rate profile for the formation of diene lactone from the *cis,cis*-3-halomuconates (Figure 3) suggests that the key to the mechanistic explanation of the reactivity of these sub-

(12) Harper,D. B.; Blakeley, E. R. *Can. J. Microbiol.* **1971**, *17*, 1015.
(13) Calculation of the isotope effect is based on the assumption that the rate of formation of diene lactone **2** is not affected by the change to deuteriated solvent. The reaction in either solvent was complete after 1 h.
(14) Elvidge, J. A.; Linstead, R. P.; Sims, P.; Orkin, B. A. *J. Chem. Soc.* **1950**, 2235.

Chart I



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strates in the pH range above pH 1 lies in the protonation state of diacid. At high pH (above pH 7), no lactonization occurs even though the attacking carboxylate exists mainly as the free anion. This suggests that the second deprotonated carboxylate group deactivates the double bond toward nucleophilic addition. Within the pH region of the pK_as of the carboxyl groups, the two tautomeric monoanions represent the dominant species. With one overall negative charge per molecule, these species are activated toward nucleophilic attack by one of the carboxylates without destabilization by the second carboxylate. This is consistent with the observed maximal rate of the diene lactone formation between pH 3 and 4 (Figure 3).

The higher rate of reaction of the 3-fluoromuconate than that of the chloride and bromide analogues found in the diene lactone formation (Figure 3) correlates with the electron-withdrawing capability of the halide substituents rather than with their leaving group ability. This is consistent with the attack of the C-6 carboxylate on carbon 3 being a rate-limiting step. The expulsion of the halide then may follow from intermediate **6** (Chart I) in a fast step. The E/Z ratio of the formed diene lactone (9/1) can be thought to reflect diastereomeric transition states for the halide expulsion arising from two rotameric forms of **6**. 4-Halo lactone **4** can be discounted as an intermediate in this reaction on grounds of the observed stability of 4-fluoromuconolactone (**4a**) toward elimination of HF even at pH 7. The lack of deuterium incorporation into the formed diene lactones further discredits **4** as an intermediate. It also discredits any mechanism involving protonation at C-5 of intermediate **6**.

When the pH is lowered to pH 3.5 and below, the cis,trans acids **3b,c** appear as additional products as is visible in Figure 1. Ainsworth and Kirby have proposed an amphoteric mechanism for the stereomutation of *cis,cis*-3-carboxymuconic acid.[7] They suggested that a free carboxylate attacks the carbon skeleton to form an intermediate reminiscent of species **6**, which after rotation about the single bond reopens to the *cis,trans*-3-carboxymuconic acid. Such an amphoteric mechanism predicts stereomutation to proceed fastest in a pH range close to the pK_a. Our results show, however, that *cis,trans*-muconic acids **3b,c** appear in increasing amounts with decreasing pH, which argues for cationic activation. The inability of *cis,cis*-3-fluoromuconate (1a) to form the cis,trans acid **3a** between pH 1 and 6 also supports the latter mechanism.

If the pH is further lowered, the overall charge of the muconate approaches zero before further protonation of the carboxyl carbonyl groups becomes significant. This state is represented by the neutral diacid. Lactonization of the latter is not significantly activated by either a deprotonated carboxyl group or a protonated carboxyl carbonyl. Hence, any reaction should be slow. The reduced extent of substrate conversion at pH 1.6 seen in the ^1H NMR analysis of the reaction of 3-halomuconates **1b,c** (Figure 1), as well as the drop off in diene lactone formation toward pH 0 (Figure 3), is in agreement with this view.

Reaction in Concentrated HCl. The prevalent features of the reaction of 3-halomuconates **1a–c** in concentrated HCl are the change in regiochemistry of lactonization of the 3-chloro- and 3-bromomuconate compared to

Scheme II

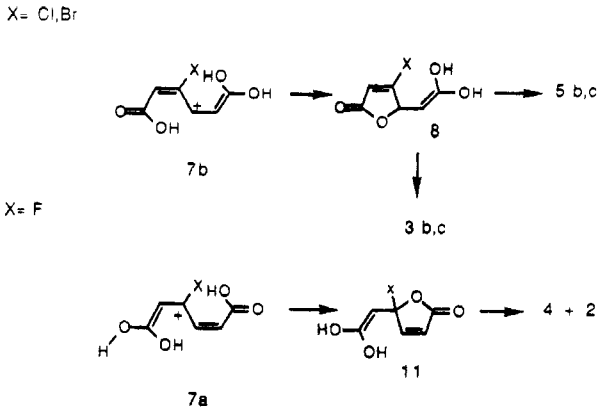
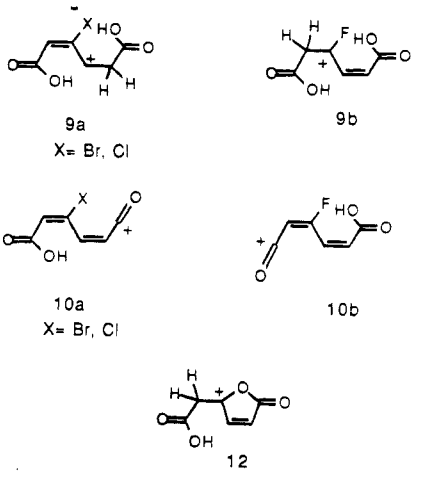


Chart II



higher pH, as well as the difference in regiochemistry between 3-fluoromuconate (**1a**) and its heavier halide analogues. The mode of isomerization at pH 0 can be explained by the difference in stability of cation **7a** versus **7b**, depending on the halide substituent (Scheme II). Indirect evidence for the viability of an O-protonated carboxyl species can be inferred from the known fast ^{18}O exchange of acids in acidic media.[15] When the C-3 halide substituent is a chloride or a bromide, then cation **7a** is destabilized compared to **7b** due to the considerable build up of positive charge character (Scheme II). Cation **7b**, on the other hand, places partial positive charge on C-4 and thus activates lactonization via the C-1 carboxyl group to yield intermediate **8** (Scheme II). This intermediate can partition to form either cis,trans acids **3b,c** or 3-halomuconolactones **5b,c**. The former can arise from rotation about the C-4,C-5 single bond in **8** with subsequent reopening of the lactone ring. While this step is analogous to the Kirby mechanism discussed above, the main distinction lies in the activation step of the lactonization. Tautomerization of **8**, on the other hand, leads to lactones **5b,c**. This mechanism is consistent with the monodeuteriation of **5b,c** at the H_5 position.

An alternate mechanism might propose carbon protonation α to the carboxyl group to give intermediate **9a** (Chart II). Such a process is well documented in the case of cis–trans isomerization of cinnamic acid in H_2SO_4.[16] It must be excluded here, however, since it would lead to incorporation of deuterium into the formed *cis,trans*-3-halomuconates **3b,c**, which is not observed. Another

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conceivable active species is the acylium ion **10a** (Chart II) that could lactonize to give a ketene intermediate. Hydration of the ketene can be expected to proceed via protonation at C-5 of **10a**[17] and would then lead to the H_5 monodeuteriated 3-halomuconolactones **5b,c**. While this explanation cannot be discounted for **5b,c**, it does not account for the formation of the nondeuteriated cis,trans acids **3b,c**.

In the case of 3-fluoromuconate (**1a**), the product mixture in concentrated HCl, concomitant formation of diene lactone **2**, and 4-fluoro lactone **4a** can be explained via the resonance stabilization that the fluoride substituent exerts on cation **7a** (Scheme II). Such stabilization via the fluoronium ion, which is well documented,[18] facilitates lactonization via the C-6 carboxylate. This leads to intermediate **11**, which via halide expulsion or tautomerization gives the observed products **2** and **4a**. Switching from HCl to DCl shows a product deuterium isotope effect of the magnitude 3.5 in favor of diene lactone **2** formation. This is consistent with the postulate that **4a** and **2** arise from the common intermediate **12**. In deuteriated solvent the tautomerization may be slowed compared to protio solvent, whereas the expulsion of the fluoride substituent is presumably less affected by this solvent change.

Carbon protonation at C-2 of the 3-fluoromuconate can again be excluded on the grounds of the observed lack of deuterium incorporation into the formed diene lactone **2**. One could in principle argue that **4a** does form via cation **9b** (Chart II) and that the observed isotope effect represents a preference for O-protonation to give **2** over C-protonation to give **4a** upon introduction of deuteriated solvent. Acylium ion **10b** presents yet another alternative for the formation of fluoro lactone **4a**, but not for the formation of **2**.

Reaction of *cis,cis*-3-Fluoromuconate in Concentrated H_2SO_4. The reaction of *cis,cis*-3-fluoromuconate (**1a**) in concentrated H_2SO_4 displayed some intriguing differences to the reaction in concentrated HCl. While the overall regiochemistry of lactonization remained the same, no 4-fluoromuconolactone (**4a**) was formed and diene lactones **2a** and **2b** were obtained in a significantly different ratio (nearly 1/1 **2a/2b** in concentrated H_2SO_4 versus 9/1 **2a/2b** in concentrated HCl). In addition the diene lactone isomers both showed 50% deuterium incorporation at the C-5 position (Figure 5). These results may be explained by competing C- and O-protonation. In addition to the O-protonation pathway discussed above for concentrated HCl (Scheme II), direct C-protonation at C-2 of **1a** leads to cation **9b** (Chart II). Lactonization of both **9b** and **7a** provides fluoro lactone **4a**, which can be ionized to the planar intermediate **12** (Chart II). The latter can collapse to the diene lactone isomers **2a** and **2b**. Thus, the extent of deuteriation in **2a,b** may reflect partitioning of **11** between tautomerization and fluoride elimination, as well as isotopic discrimination in the proton loss from **12**. Solvent exchange of H_5 of **4a** and of **2a,b** can be excluded since it would predict a higher than observed deuterium incorporation.

Conclusion

We have demonstrated here the different modes of reaction of the title compounds in three different acidic media (aqueous buffer of pH 1–6, concentrated HCl and concentrated H_2SO_4). These findings, along with the proposed explanations, might be useful in the interpretation of results from metabolic studies of haloaromatic compounds, where these compounds are involved.[1-6,11] In addition, we provide here an example of an acid-induced reaction proceeding differently in concentrated HCl than in the highly ionizing medium concentrated H_2SO_4. Thus, observations made in HCl are not readily transferrable to H_2SO_4 and vice versa.

Experimental Section

Materials. Reagents and buffer salts were of analytical grade and were used as received. Doubly distilled water was used for the preparation of reaction media. Deuteriated solutions were prepared with 99.8 atom % D_2O (Aldrich). Deuteriated buffer salts were prepared by repeated (four times) exchange with D_2O. The synthesis of 3-halomuconates **1a–c** is described in a previous publication.[5]

Identification of Reaction Products. ^1H NMR spectra were recorded on an IBM AM400 or AF200 spectrometer. Low-resolution and high-resolution mass spectra were recorded on a VG 7070E spectrometer. Ultraviolet spectra and absorbance changes were measured on a Gilford response II spectrometer.

Isomerization of *cis,cis*-3-Halomuconates 1a–c between pH 1–6. The 3-halomuconate (3 mg) was dissolved at time = t_0 in buffer (100 mM KD_2PO_4, D_2O, 1 mL), which was previously adjusted with either dilute DCl or NaOD to the desired pH (pH 8.0, 5.4, 3.2, 2.5, 1.1). After 1 h the product mixture was analyzed by ^1H NMR. The data for the reaction of 3-bromomuconate are shown in Figure 1. The relative integrations of reaction products are given in Table I. The results for the reaction of 3-chloromuconate under these conditions are the same. Above pH 3.2 the product mixture from isomerization of 3-fluoromuconate (**1a**) was the same as observed for the chloride and bromide analogues (Figure 1). At pH 1.1 the product mixture from exposure of **1a** to the reaction media for 1 h showed following ^1H NMR spectrum. ^1H NMR (100 mM KD_2PO_4, D_2O, 200 MHz): δ 8.18 (d, **2a**, J = 5.7 Hz), 7.58 (d, **2b**, J = 5.6 Hz), 7.01 (dd, **1a**, J = 28.6 Hz, J = 12.5 Hz), 6.45 (dd, **2a**, J = 5.7 Hz, J = 0.6 Hz), 6.43 (d, **2b**, J = 5.6 Hz), 6.05 (d, **1a**, J = 12.5 Hz), 5.86 (d, **2a**, J = 0.6 Hz), 5.69 (d, **1a**, J = 19.0 Hz), 5.56 (s, **2b**). Integration of the ^1H NMR spectrum gave a ratio of **2a/2b/1a** of 74%/13%/13%. The assignments of **2a** and **2b**[6] and of **1a**[9] agree with values given in the literature.

pH–Rate Profile of the Cycloisomerization of 3-Halomuconates 1a–c. The concentrations of stock solutions of **1a–c** were determined with reference to extinction coefficients given in the literature.[3] The buffer solutions (50 mM KH_2PO_4) were adjusted to the desired pH values (pH 6.5, 5.1, 4.1, 3.6, 3.0, 1.6) with dilute HCl or NaOH. The pH 0.25 buffer consisted of 1 N H_2SO_4. Reaction mixtures (1-mL volume) contained 37 nmol of 3-halomuconate. The increase in absorbance at 277 nm was monitored over 3 min. The values of absorbance change over time used for rate calculations were the means of five runs; the largest standard deviation was 12.9%. Rates of formation of diene lactone **2** were calculated with extinction coefficients of authentic material in each pH buffer. The results are shown in Figure 2.

General Procedure for the Isomerization of *cis,cis*-3-Halomuconates 1a–c in Concentrated Acids. The *cis,cis*-3-halomuconate (typically 30 mg) was stirred with acid (HCl or DCl, 5 mL) at room temperature for 1 h. The mixture was evaporated to dryness and analyzed by ^1H NMR. 3-Halomuconolactones **5b,c** were separated from *cis,trans*-3-halomuconic acids **3b,c** by taking up the product mixture in water and filtering the precipitated cis,trans acids off. The water layer was then evaporated to yield the 3-halomuconolactones. These were analyzed by mass spectrometry. The product mixture from the isomerization of 3-fluoromuconate (**1a**) at pH 0 was analyzed without further separation. Reactions run in H_2SO_4 were worked up by dilution with an equal volume water and extracted with ether.

Product Mixture from the Isomerization of *cis,cis*-3-Fluoromuconate (1a) in HCl. ^1H NMR (D_2O, 200 MHz): δ 8.25 (d, **2a**, J = 5.7 Hz), 7.61 (d, **4a**, J = 5.7 Hz), 7.52 (d, **2b**, J = 5.7 Hz), 6.53 (d, **2a**, J = 5.7 Hz), 6.50 (d, **2b**, J = 5.7 Hz), 6.36 (dd, **4a**, J = 5.7 Hz, J = 1.6 Hz), 3.26 (m, **4a**). Integration of the ^1H NMR spectrum gave a ratio of **2a/2b/4a** of 21%/2%/77%. EI

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mass spectrum, m/e: 160 (M^+, **4a**), 140 (M^+ – HF, **4a**), 112 (M^+ – HF – CO, **4a**), 101 (M^+ – CH_2COOH, **4a**), 73 (M^+ – CH_2COOH – CO, **4a**). HRMS (m/e) for $C_6H_5FO_4$: Calcd 160.0172, found 160.0173.

Product Mixture from Isomerization of *cis,cis*-3-Fluoromuconate (1a) in DCl. ^1H NMR (D_2O, 200 MHz): δ 8.24 (d, **2a**, J = 5.7 Hz), 7.59 (d, **4a**, J = 5.7 Hz), 7.51 (d, **2b**, J = 5.7 Hz), 6.51 (d, **2a**, J = 5.7 Hz), 6.48 (d, **2b**, J = 5.7 Hz), 6.35 (d, **4a**, J = 5.7 Hz), 5.92 (s, **2a**), 5.61 (s, **2b**), 3.2 (m, **4a**). Integration of the ^1H NMR spectrum gave a ratio of **2a**/**2b**/**4a** of 40%/11%/49%.

Product Mixture from Isomerization of *cis,cis*-3-Chloromuconate (1b) in HCl. ^1H NMR (methanol-d_4, 200 MHz): δ 8.59 (dd, **3b**, J = 0.6 Hz, J = 15.4 Hz), 6.49 (dd, **3b**, J = 0.6 Hz, J = 15.4 Hz), 6.39 (t, **3b**, J = 0.6 Hz), 6.36 (d, **5b**, J = 1.8 Hz), 5.44 (m, **5b**), 3.08 (dd, **5b**, J = 3.5 Hz, J = 16.8 Hz), 2.66 (dd, **5b**, J = 16.8 Hz, J = 7.9 Hz). Integration of the ^1H NMR spectrum gave a ratio of **5b**/**3b** of 88%/12%. Integration of the ^1H NMR spectrum of the reaction run in DCl showed only deuterium incorporation into the C-5 position of **5b**. EI mass spectrum of 3-chloromuconolactone (**5b**), m/e: 178 (M + 2), 176 (M^+), 160 (M + 2 – H_2O), 158 (M^+ – H_2O), 140 (M^+ – Cl), 132 (M + 2 – HCOOH), 130 (M^+ – HCOOH), 119 (M + 2 – CH_2COOH), 117 (M^+ – CH_2COOH). HRMS (m/e) of 3-chloromuconolactone (**5b**) ($C_6H_5ClO_4$): calcd 175.9876, found 175.9877.

Product Mixture from Isomerization of *cis,cis*-3-Bromomuconate (1c) in HCl. ^1H NMR (methanol-d_4, 200 MHz): δ 8.51 (dd, **3c**, J = 15.1 Hz, J = 0.9 Hz), 6.65 (dd, **3c**, J = 0.7 Hz, J = 0.9 H), 6.52 (d, **5c**, J = 1.8 Hz), 6.44 (dd, **3c**, J = 15.1 Hz, J = 0.7 Hz), 5.46 (m, **5c**), 3.09 (dd, **5c**, J = 16.8 Hz, J = 3.6 Hz), 2.63 (dd, **5c**, J = 16.8 Hz, J = 7.9 Hz). Integration of the ^1H NMR spectrum gave a ratio of **5c**/**3c** of 92%/2%. Integration of the ^1H NMR spectrum of the reaction run in DCl showed only deuterium incorporation into the C-5 position of **5c**. EI mass spectrum of 3-bromomuconolactone (**5c**), m/e: 204 (M + 2 – H_2O), 202 (M^+ – H_2O), 177 (M + 2 – COOH), 176 (M + 2 – HCOOH), 175 (M^+ – COOH), 174 (M^+ – HCOOH), 163 (M + 2 – CH_2COOH), 161 (M^+ – CH_2COOH), 141 (M^+ – Br). HRMS (m/e) of 3-bromomuconolactone (**5c**) ($C_5H_4BrO_2$ (M^+ – COOH)), calcd 174.9395, found 174.9396.

Stability of 4-Fluoromuconolactone (4a) at pH 7.0. A sample of 4-fluoromuconolactone (**4a**) (2 mg) was dissolved in the pH buffer (100 mM KD_2PO_4, D_2O, pH 7.0, 1 mL) in an NMR tube. The mixture was monitored by ^1H NMR for 4 h. No change in the initial spectrum was observed. ^1H NMR (pH buffer, 200 MHz): δ 7.87 (d, 1 h, J = 5.7 Hz), 6.58 (d, 1 h, J = 5.7 Hz), 3.30 (m, 1 h), 3.16 (m, 1 h).

Isomerization of *cis,cis*-3-Fluoromuconate (1a) in Concentrated H_2SO_4. *cis,cis*-3-Fluoromuconate (**1a**) (30 mg) was dissolved in concentrated H_2SO_4 (5 mL) and the resultant mixture stirred at room temperature for 1 h. An equal volume of ice water was added, and the mixture was extracted with ether. Evaporation of the organic phase yielded the product mixture, which was analyzed by ^1H NMR. ^1H NMR (D_2O, 200 MHz): δ 8.24 (d, **2a**, J = 5.7 Hz), 7.51 (d, **2b**, J = 5.7 Hz), 6.51 (d, **2a**, J = 5.7 Hz), 6.48 (d, **2b**, J = 5.7 Hz), 5.92 (s, **2a**), 5.61 (s, **2b**). Integration of the ^1H NMR spectrum gave a ratio of **2a**/**2b** of 56%/44%. Isomerization of **1a** in concentrated D_2SO_4 was carried out as described for concentrated H_2SO_4. The chemical shift values of the resonances of **2a** and **2b** were identical (Figure 5). Integration of the ^1H NMR spectrum gave a ratio of **2a**/**2b** of 58%/42%. The resonance corresponding to H_5 in **2a** and **2b** showed 50% deuterium incorporation.

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Unique Single-Electron Transfers between Chemically Inert Triphenylmethyl Radicals and Triphenylmethyl Anions

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A number of SET reactions between inert 4-X-tetradecachlorotriphenylmethyl radicals (X-PTM$^\bullet$) and stable tetra-*n*-butylammonium (Q^+) 4-Y-tetradecachlorotriphenylmethides (Q^+ Y-PTM:$^-$; X, Y ≡ H, Me, NH_2CO, Me_2NCO, Ph_2NCO, MeOCO, PhOCO, NH_2, MeO, Cl, Br, ^-OCO) have been studied in THF, at room temperature, by the ESR technique. These processes are abnormally slow, since no significant ESR linewidth and/or hyperfine coupling changes are observed. In the SET between H-PTM$^\bullet$ and NH_2-PTM:$^-$ the progress has been monitored by ESR, and its second-order rate constant is 5×10^2 mol^{-1} L min^{-1}. Such a unique, most remarkable slowness is ascribed to colossal steric hindrance (shielding) caused by chlorine overcrowding in both SET components. The SET equilibrium constants K_{ET} have been calculated from the ESR spectrum, using radicals H-PTM$^\bullet$ and Me-PTM$^\bullet$ as the standards, and they follow the Hammett equation. Exceptions are X = MeO or RCO, due to steric inhibition of resonance. Evidence indicates that the SET process occurs between the radical and the free carbanion, in spite the latter existing predominantly as an ion pair with counterion Q^+, as shown by osmometry. The syntheses and isolation in excellent yields of a substantial number of new inert free radicals and new related stable carbanion salts have been effected, most of the latter from the corresponding radicals, using hydroxide ion as a single-electron donor.

Single-electron transfer (SET) is a fundamental process in chemistry. Although many organic reactions involve it, on account of the high reactivity and instability of the free-radical species involved as starting components, intermediates, or final products, those that allow a deep insight and a straightforward, unambiguous interpretation of the relevant phenomena are rather scarce.

An outstanding SET class occurs among carbanions, carbenium ions, and trivalent carbon free radicals. Unfortunately, research work on it is dramatically lacking because of the unavailability of both ionic and radical species provided with sufficient stability and low reactivity in non-SET processes. Spectral evidence for a reversible SET between 4,4′-bis(dimethylamino)triphenylcarbenium ion and 4,4′,4″-trinitrotriphenylcarbanion, giving their free radicals, has been reported.[1] The reaction of equivalent amounts of perchlorotriphenylmethyl cation, $(C_6Cl_5)_3C^+$,

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