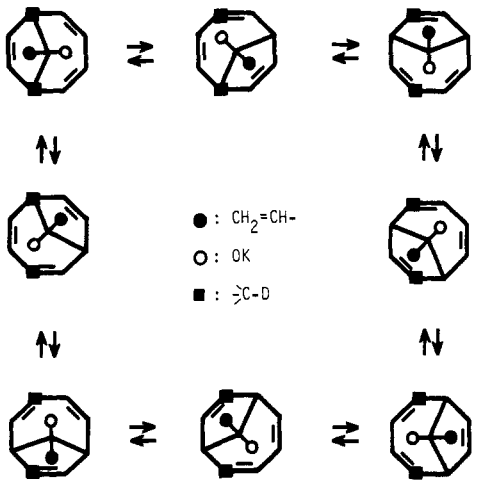
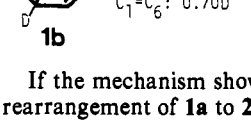


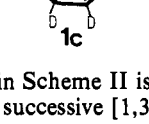
Figure 2. Circumambulatory [1,3]-sigmatropic rearrangement of **1b** with inversion of configuration in each step.

to a mixture composed of the same products. On the other hand, **9** afforded **4** as the major product along with a mixture of four vinyl alcohols as the minor products.

Although the formation of detectable amounts of **9** was not clearly observed except in the preparative scale experiments because of its very low accumulation, it is noteworthy that all vinyl alcohols afforded the same products. Moreover, evidence that neigher **5** nor **6**, direct [1,3]-sigmatropic rearranged ketones from **7** and **9**, was formed from any vinyl alcohols is rather surprising if ketone **4** were directly formed from **3** and **8**. Thus, a plausible mechanism for the formation of **4** from **3**, **7**, and **8** can be proposed as shown in Scheme II, where **4** is formed in the indirect mechanism through **9** which is in equilibrium with **3**, **7**, and **8** under the conditions employed. In order to gain further insight into the interrelation among vinyl alcohols, reactions and kinetic studies without 18-crown-6 were carried out, expecting suppression of high energy pathways of these four [1,3]-sigmatropic pathways. Below 49.5 °C, the rapid equilibrium between **3** and **7** was only observed. For instance, at 49.5 °C, both **3** and **7** equilibrated each other within 15 min, and the formation of the equilibrium mixture of **3**, **7**, and **8** required prolonged heating for more than 5 h, while the formation of **4** was suppressed even upon prolonged heating of a mixture of **3**, **7**, and **8** at 64.5 °C. This likely suggests that the addition of 18-crown-6[9] significantly accelerates high-energy pathways such as the more sterically unfavorable inversion pathway from **8** to **9** as compared with those between **3** and **7** and/or the energetically unfavorable retention pathway from **3** to **9** included in the indirect mechanism. Thus, the following activation parameters were obtained for the interconversions between **3** and **7** (20.2–49.5 °C) and between **7** and **8** (41.0–64.5 °C) without 18-crown-6, providing the first detection of the symmetry-forbidden anionic [1,3]-sigmatropic retention pathways[14] between **7** and **8** which compete with the inversion pathway from **7** to **3** in the rate ratio k_{ret}/k_{inv} = 1/82.2 at 49.5 °C; E_a = 19.9 kcal/mol (log A = 11.6) for **3** → **7**; E_a = 18.8 kcal/mol (log A = 10.7) for **7** → **3**; E_a = 20.6 kcal/mol (log A = 10.0) for **7** → **8**; E_a = 22.3 kcal/mol (log A = 11.2) for **8** → **7**.



1b C_1=C_6: 0.70D



1c

C_1=C_6: (0.26±0.02)D
C_2=C_5: (0.15±0.01)D
C_3=C_4: (0.13±0.02)D
C_7=C_8: (0.15±0.01)D

If the mechanism shown in Scheme II is correct, during the rearrangement of **1a** to **2** by successive [1,3]-, with retention of configuration, and [3,3]-sigmatropic rearrangements, the competitive [1,3]-sigmatropic rearrangement with inversion of configuration must involve the circumambulation of the C_9 carbon of **1a** which can be seen when **1b**[15] is used. The recovered vinyl alcohol **1c**[17] isolated after treating **1b** with KH at 20 °C for 11 min unequivocally indicated the occurrence of the circumambulatory [1,3]-sigmatropic rearrangement with inversion of configuration in each step as shown in Figure 2, supplementing the operation of a consecutive mechanism.

Registry No. 1a, 80434-45-3; **2**, 80434-46-4; **3**, 80434-47-5; **4**, 80434-48-6; **7**, 80434-49-7; **8**, 80482-66-2; **9**, 80447-41-2.

(14) The symmetry-forbidden [1,3]-sigmatropic rearrangement with retention of configuration has been detected in pyrolyses of several "neutral" systems and discussed in detail by Berson. See J. A. Berson, *Acc. Chem. Res.*, **5**, 406 (1972), and references cited therein.

(15) Compound **1b** was prepared according to the procedure[16] reported by Paquette. Bicyclo[4.3.0]nona-2,4-dien-8-one was deuteriated with $NaOCH_3/CH_3OD$. Successive bromination, dehydrobromination, and the addition of CH_2=CHLi gave **1b**.

(16) L. A. Paquette, R. H. Meisinger, and R. E. Wingard, Jr., *J. Am. Chem. Soc.*, **94**, 2155 (1972).

(17) The distribution of deuteriums in **1c** was obtained from Eu(fod)$_3$ pseudocontact ^{1}H NMR spectra.

Enzymic Reduction of an Epoxide to an Alcohol

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We wish to report the first example of direct enzymic reduction of an epoxide to an alcohol.[1,2] Incubation of 24(*R*),25-oxidolanosterol (**1**) with standard S_{10} rat liver homogenate (RLH)[3] results in formation of 24(*R*)-hydroxycholesterol (**2**). Evidence is presented below which indicates that this transformation does not occur via an intermediate 24-keto steroid.[2]

In connection with our previous demonstration[4] that squalene 2,3(*S*);22(*S*),23-dioxide is converted efficiently by RLH to 24(*S*),25-epoxycholesterol (**3**), we wished to establish that 24(*S*),25-oxidolanosterol (**4**) would also be converted by RLH to **3**. Preparation of [2-^{3}H]**4** required separation by preparative TLC[5] of a mixture of the known[6] acetates of [2-^{3}H]**4** and [2-^{3}H]**1**[7] followed by saponification. Incubation of the [2-^{3}H]**4** with RLH for 30 min afforded 18% of product with the TLC[5] R_f value of **3**, plus 45% of unreacted **4**. The identity of **3** was confirmed, as before,[4] by $LiAlH_4$ reduction to 25-hydroxycholesterol (**5**), isotopic

(1) Dixon and Webb (Dixon, M.; Webb, E. C. "Enzymes", 3rd ed.; Academic Press: New York, 1979) list no enzyme which effects this conversion.

(2) Siekmann, Disse, and Breuer (Siekmann, L.; Disse, B.; Breuer, H. *J. Steroid Biochem.* **1980**, *13*, 1181-1205) claim that incubation of 16α,17-epoxyprogesterone (i) with rat liver microsomes affords 5% of 16β-hydroxyprogesterone (ii) and suggest that this conversion proceeds via 16-oxoprogesterone; i → ii obviously cannot be a direct epoxide reduction.

(3) RLH was prepared according to Popjak (Popjak, G. *Methods Enzymol.* **1969**, *15*, 438–440). Incubations were conducted as follows. To 5.0 mL of S_{10} RLH, without addition of coenzymes, was added 25–55 μg of purified ^{3}H-labeled substrate (specific activity ca. 38 000 dpm/μg) dissolved in ca. 50 μL of an aqueous solution containing 70–100 mg per mL of Triton WR 1339 (Ruger Chemical, Irvington, NJ). The resulting mixture was incubated at 37 °C for 60 min unless a different length of time is specified in the text. All incubations were run at least in duplicate.

(4) Nelson, J. A.; Steckbeck, S. R.; Spencer, T. A. *J. Biol. Chem.* **1981**, *256*, 1067–1068.

(5) TLC analyses were performed on LK5D silica gel plates (Whatman, Inc., Clifton, NJ); preparative TLC plates were prepared with Silica Gel 60 PF 254 + 366 (EM Laboratories, Inc., Elmsford, NY). Various ratios of ether–hexane were employed as eluent, unless noted otherwise.

(6) Boar, R. B.; Lewis, D. A.; McGhie, J. F. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2231–2235.

(7) [2-^{3}H]Lanosterol, specific activity = 38 000 dpm/μg, prepared by treatment of the corresponding ketone with acidic tritium oxide in THF, by the method of Nadeau and Hanzlik (Nadeau, R. G.; Hanzlik, R. P. Reference 3, pp 346–349) was converted to the mixture of acetates of [2-^{3}H]**1** and [2-^{3}H]**4** by the procedure given in ref 6.

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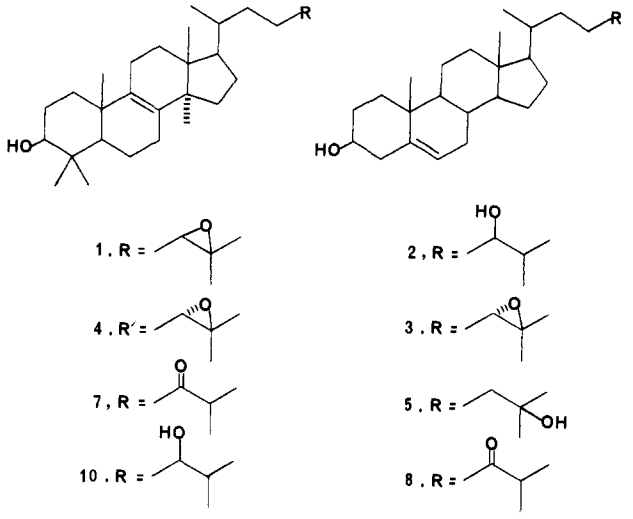
dilution with unlabeled **5**,[8] and recrystallization to constant specific activity[9] as well as by preparation of a benzoate derivative and TLC comparison with known[10] 24(*S*),25-epoxycholesterol benzoate.



Having [2-^{3}H]24(*R*),25-oxidolanosterol (**1**) in hand, we decided to see whether 24(*R*),25-epoxycholesterol would be formed from **1** by RLH. When [2-^{3}H]**1** was incubated for 30 min in the same RLH used with [2-^{3}H]**4**, the results were strikingly different. Essentially no **1** was recovered. TLC analysis showed two major product fractions, containing 23% and 18% of the ^{3}H, respectively. The latter, more polar product had an R_f value very similar to, but slightly greater than, that of 25-hydroxycholesterol (**5**). Evidence that this more polar product did not contain an oxirane ring was obtained when its TLC behavior was unchanged after treatment with $LiAlH_4$ in ether for 22 h. These observations led us to consider that this product might be **2**.

Pure 24(*R*)-hydroxycholesterol (**2**) and its dibenzoate derivative **6** have been prepared by Ikekawa and co-workers.[11] We prepared **6** by a different route, involving BF_3·Et_2O rearrangement of a mixture of 24(*R*)- and 24(*S*),25-epoxycholesterol benzoates[10] to 24-ketocholesterol benzoate[12] followed by $NaBH_4$ reduction to a mixture of 24(*R*)- and 24(*S*)-hydroxycholesterol benzoates[12] and treatment with benzoyl chloride and pyridine to afford **6** and its 24*S* epimer, which were separated by preparative TLC.[13]

The more polar incubation product was benzoylated, diluted with **6**, and recrystallized to constant specific activity.[14] The identity of the incubation product as 24(*R*)-hydroxycholesterol (**2**) was established. That the formation of **2** had occurred biochemically was indicated by the essentially quantitative recovery of **1** when it was incubated with a boiled RLH preparation.

A plausible mechanistic hypothesis[2] for the oxirane reduction which occurs during **1** → **2** consists in acid-catalyzed rearrangement to a 24-keto steroid followed by stereoselective reduction to **2**. To test this proposal, [2-^{3}H]24-ketolanosterol (**7**) was prepared by BF_3·Et_2O treatment[15] of the mixture of the acetates of [2-^{3}H]**1** and [2-^{3}H]**4** followed by saponification. Incubation of this [2-^{3}H]**7** with RLH gave no detectable 24-(*R*)-hydroxycholesterol (**2**). There was formed instead 15% of 24-ketocholesterol (**8**), which was identified by conversion to its benzoate (**9**), isotopic dilution with known[12] unlabeled **9**, and recrystallization to constant specific activity.[16] Incubation of a mixture of **1** and **4** with this same RLH resulted in the formation of 13% **2** and 19% **3** but no **8**. These results strongly suggest that a 24-keto steroid is not an intermediate in the biochemical conversion of **1** to **2**.

Our current hypothesis is that the enzymic epoxide reduction is related to the reduction of the $\Delta^{24,25}$ double bond in the latter stages of cholesterol biosynthesis. This has been shown to involve addition of one hydrogen from H_2O at C-24 and one from NADPH at C-25.[17] It is also known that the introduction of these hydrogens is cis, with the incoming H at C-24 in the pro-*S* position.[18] These results require that if 24(*R*),25-oxidolanosterol (**1**) were enzymically bound in the same manner as a $\Delta^{24,25}$-steroid, the back side of the C–O bond at C-25 would be accessible to attack by NADPH. Conversely, if 24(*S*),25-oxidolanosterol (**4**) were so bound, the epoxide oxygen would protrude toward the NADPH, and hydride transfer to C-25 could not occur.

It has been shown[19] that reduction of the $\Delta^{24,25}$ double bond in lanosterol occurs in RLH in the absence of oxygen. To see whether the oxirane ring of **1** would suffer the same type of reduction, a mixture of [2-^{3}H]**1** and [2-^{3}H]**4** was incubated in RLH under N_2. The nonsaponifiable extract[3] from this incubation contained 39% of material with unchanged R_f and 35% with the R_f of 24(*R*)-hydroxylanosterol (**10**).[6] After these products were separated by preparative TLC, it was determined that the recovered oxidolanosterol was **4** by demonstration that its acetate derivative had the R_f value of authentic 24(*S*),25-oxidolanosterol acetate[6] rather than that of its *R* epimer.[6] The other product was conclusively identified as **10** by dilution with authentic unlabeled **10**[6] and recrystallization to constant specific activity.[20,21]

Finally, since there is a reported case of epoxide reduction by NADPH in the absence of enzyme,[22] a mixture of [2-^{3}H]**1** and [2-^{3}H]**4** was incubated under the usual conditions[3] with NADPH[23] in the absence of RLH. No 24(*R*)-hydroxylanosterol (**10**) was formed, and the reactants were quantitatively recovered. It is thus evident that the oxirane ring of **1** is enzymically reduced by RLH under either aerobic or anaerobic conditions. Because 24-ketolanosterol (**7**) is not reduced to **10** but is instead converted to 24-ketocholesterol (**8**), it seems extremely unlikely that a 24-keto steroid is an intermediate in this reduction.[24]

Our investigation of the biochemistry of squalene 2,3(*S*);22-(*S*),23-dioxide was originally undertaken to determine if this substance might be a precursor of 25-hydroxycholesterol (**5**), which is a known inhibitor of HMG-CoA reductase and has been suggested to be part of the natural mechanism of cholesterol regulation.[25] As noted earlier, we found that this dioxide is efficiently

(8) Steraloids, Inc., Wilton, NH.
(9) Initial specific activity = 2440 dpm/mg. Recrystallization from methanol yielded **5** with, successively, 2520 and 2490 dpm/mg.
(10) Seki, M.; Koizumi, N.; Morisaki, M.; Ikekawa, N. *Tetrahedron Lett.* **1975**, 15–18.
(11) Koizumi, N.; Morisaki, M.; Ikekawa, N. *Tetrahedron Lett.* **1975**, 2203–2206.
(12) Ronchetti, F.; Russo, G. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1823–1825. These workers reported, but did not characterize, 24-ketocholesterol benzoate (**9**), for which we have obtained mp 181.5–183.5 °C and correct combustion analysis.
(13) Separation of **6** from its 24*S* epimer required about 10 elutions with 3:7 methylene chloride–hexane.
(14) Initial specific activity = 2500 dpm/mg. Recrystallization from ether–methanol yielded **6** with, successively, 1640, 1460, 1400, and 1410 dpm/mg.
(15) Barton, D. H. R.; Harrison, D. M.; Moss, G. P.; Widdowson, D. A. *J. Chem. Soc. C* **1970**, 775–785. Briggs, L. H.; Bartley, J. P.; Rutledge, P. S. *J. Chem. Soc., Perkin Trans. 1* **1973**, 806–809.
(16) Initial specific activity = 3915 dpm/mg. Recrystallization from ether yielded **9** with, successively, 3810, 3690, and 3790 dpm/mg.
(17) Akhtar, M.; Munday, K. A., Rahimtula, A. D.; Watkinson, I. A.; Wilton, D. C. *J. Chem. Soc., Chem. Commun.* **1969**, 1287–1288.
(18) Greig, J. B.; Varma, K. R., Caspi, E. *J. Am. Chem. Soc.* **1971**, *93*, 760–766. Kienle, M. G.; Varma, R. K.; Mulheirn, L. J.; Yagen, B.; Caspi, E. *Ibid.* **1973**, *95*, 1996–2001.
(19) Avigan, J.; Goodman, D. S.; Steinberg, D. *J. Biol. Chem.* **1963**, *238*, 1283–1286.
(20) Initial specific activity = 7510 dpm/mg. Recrystallization from acetone–ethanol yielded **10** with, successively, 6590, 6500, 6370, and 6460 dpm/mg.
(21) TLC analysis showed that **10** had essentially the same R_f value as the other product (23%) obtained from the aerobic incubation of **1**, except that the incubation product was somewhat spread out, as if it contained **10** plus intermediates partially converted to **2**.
(22) Yang and Gelboin (Yang, S. K.; Gelboin, H. V. *Cancer Res.* **1976**, *36*, 4185–4189) have shown that NADPH, without any involvement of an enzyme, will reduce *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo-[*a*]pyrene to a triol.
(23) Sigma Chemical Co., St. Louis, MO.
(24) It is conceivable, however, that **7** is accepted as a substrate by all the RLH enzymes necessary to effect its conversion to **8** but that it is not accepted as a substrate for reduction to **2** unless it had been formed from previously enzymically bound **1**.

converted to 24(*S*),25-epoxycholesterol (**3**),[4] which we now know to be a natural product of mammalian steroid biosynthesis,[26] but that **3**, at least in RLH, is not reduced to **5**.[4] The enzymic conversion of epoxide to alcohol which we sought to detect occurs instead with 24,25-epoxy steroids having the unnatural 24*R* configuration. Further investigation of this novel facet of enzyme chemistry will include the use of isotopically labeled NADPH to determine whether that reagent is indeed the source of the hydrogen introduced at C-25. It will also be of interest to see whether an inhibitor of the reduction of the $\Delta^{24,25}$ double bond, such as triparanol,[27] will inhibit reduction of the 24(*R*),25-epoxide.

Acknowledgment. This research was supported by NIH Grant HL 23083. We thank Dr. D. J. Hupe for valuable discussions.

(25) Kandutsch, A. A.; Chen, H. W.; Heiniger, H.-J. *Science (Washington, D.C.)* **1978**, *201*, 498-501. Gibbons, G. F.; Pullinger, C. R.; Chen, H. W.; Cavenee, W. K.; Kandutsch, A. A. *J. Biol. Chem.* **1980**, *255*, 395-400.
(26) Nelson, J. A.; Steckbeck, S. R.; Spencer, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 6974-6975.
(27) Blohm, T. R.; MacKenzie, R. D. *Arch. Biochem. Biophys.* **1959**, *85*, 245-249. Reference 19 reports that triparanol is an effective inhibitor of the $\Delta^{24,25}$ reduction in RLH.

The pK_a of Acetone: A Kinetic Method for Determining the pK_as of Ketones in Aqueous Solution

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The enolization of simple ketones has long been recognized as mechanistically important and has been extensively studied.[2] Recently we reported some investigations concerning the equilibrium constants for such enolization.[3-5] In the course of these studies we realized[4] that implicit in some pioneering studies by Bartlett[6,7] was a potential method for determining the pK_a of simple ketones in water. Such determinations have not been possible by any direct method because simple ketones are far less acidic than water itself, and the available indirect methods,[8] although they have led to values which are widely accepted,[9] are necessarily lacking in rigor. Bordwell et al.[10] have determined pK_a values for simple ketones by direct methods in dipolar aprotic solvents, but there is a large and unknown solvent shift in pK_a on going from dipolar aprotic solvent to water[11] so that these values do not constitute a solution to the problem for aqueous solution. Wirz has reported[11a] a flash spectrophotometric method which gives enol pK_as and might give pK_as of ketones with a suitable chromophore; thus far it has only been applied to acetophenone.

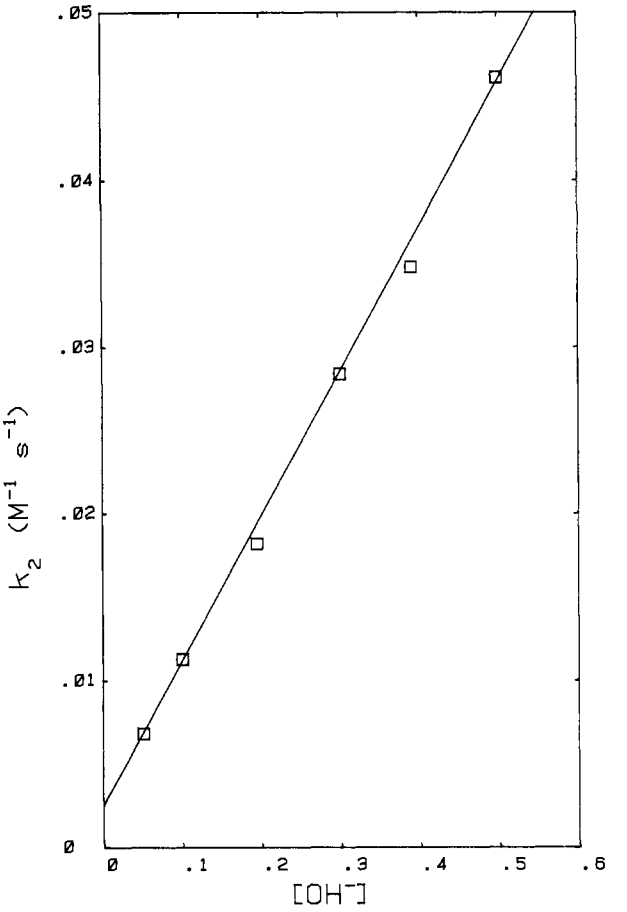

Figure 1. Second-order rate constants for the reaction of acetone with hypochlorite as a function of hydroxide concentration. Reactions were carried out with $[\text{acetone}]_0 \simeq 3 \times 10^{-4}$ M and $[OCl^-]_0 \simeq 5 \times 10^{-3}$ M. The line was fitted by least squares: $k_2 = 0.0025 \pm 0.0007 + (0.0869 \pm 0.0033)[OH^-]$.

Bartlett and Vincent[7] discovered that in alkaline solution the rate of chlorination of ketones was first order in ketone and first order in hypochlorite (in alkaline solution Cl_2 is essentially completely converted to Cl^- plus OCl^-[12]) and of mixed zero and first order in hydroxide. The term in the rate law first order in hydroxide is simply interpreted as the reaction of enolate ion with OCl^-. The term in the rate law zero order in hydroxide is kinetically ambiguous; it could represent either reaction of the enol with OCl^- or reaction of the enolate with HOCl. Since the slope and intercepts of plots of apparent second-order rate constants against hydroxide concentration are of comparable magnitude, and the enol should be much less reactive than the enolate toward a relatively feeble halogenating agent such as OCl^-, the first possibility appears very improbable.[13] Since it is known that enols react with halogens at diffusion-controlled rates,[14] and that enolates are more reactive than enols, and since it appears probable that HOCl will not be drastically less reactive than Cl_2, it seemed plausible to propose that the reaction of enolate with HOCl will also be diffusion controlled. In support of this speculation, it should be recalled that Bell and Yates,[15a] found that for diethyl malonate,

(1) E. W. R. Steacie Fellow, 1980-1982.
(2) Forsen, S.; Nilsson, M. In "The Chemistry of the Carbonyl Group"; Zabicky, J., Ed.; Wiley: London, 1970; Vol. 2, p 157.
(3) Guthrie, J. P.; Cullimore, P. A. *Can. J. Chem.* **1979**, *57*, 240.
(4) Guthrie, J. P. *Can. J. Chem.* **1979**, *57*, 797.
(5) Guthrie, J. P. *Can. J. Chem.* **1979**, *57*, 1177.
(6) Bartlett, P. D. *J. Am. Chem. Soc.* **1934**, *56*, 967.
(7) Bartlett, P. D.; Vincent, J. R. *J. Am. Chem. Soc.* **1935**, *57*, 1596.
(8) Bell, R. P. *Trans. Faraday Soc.* **1943**, *39*, 253. Pearson, R. G.; Dillon, R. L. *J. Am. Chem. Soc.* **1953**, *75*, 2439. Bell, R. P.; Smith, P. W. *J. Chem. Soc. B* **1966**, 241.
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(10) Matthews, W. S.; Bares, J. E.; Bartmess, J. E.; Bordwell, F. G.; Cornforth, F. J.; Drucker, G. E.; Margolin, Z.; McCallum, R. J.; McCollum, G. J.; Vanier, N. R. *J. Am. Chem. Soc.* **1975**, *97*, 7006.
(11) (a) Ritchie, C. D. In "Solute-Solvent Interactions"; Coetzee, J. F., Ritchie, C. D., Eds.; Marcel Dekker: New York, 1969; Vol. 1, p 229. (b) Haspra, P.; Sutter, A.; Wirz, J. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 617.
(12) Downs, A. J.; Adams, C. J. In "Comprehensive Inorganic Chemistry"; Bailar, J. C., Jr., Emeleus, H. J., Nyholm, Sir R., Trotman-Dickenson, A. F., Eds.; Pergamon: Oxford, 1973; p 1191.
(13) If the intercept were due to reaction of enol with OCl-, the microscopic rate constant would have to be comparable to or even larger than the microscopic rate constant for reaction of the enolate with OCl^-: this seems unlikely.
(14) Toullec, J.; Dubois, J. E. *Tetrahedron* **1973**, *29*, 2851. Dubois, J. E.; Toullec, J. *Ibid.* **1973**, *29*, 2859.
(15) (a) Bell, R. P.; Yates, K. *J. Chem. Soc.* **1962**, 2285. (b) Kinetics were followed spectrophotometrically, at 292 nm for OCl^- and at 331 nm for OBr^-. Glass distilled water was used for all solutions; the stability of hypohalite solutions was demonstrated before starting a run. The concentrations of hydroxide and hypohalite ions were determined by titration.

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