(lit.[21] $[\alpha]_D$ +36 (c 0.95)). IR ($CHCl_3$): ν 3600–3100, 1780, 1736, 1619, 1521 cm^{-1}. ^{13}C NMR (DMSO-d_6) δ 177.7, 173.2, 156.6, 131.4, 127.8, 115.0, 80.3, 79.7, 75.9, 69.1, 62.3, 51.5, 47.6, 32.7. MS (70 eV), m/e (relative intensity): 324 (14.4), 296 (1.6), 265 (0.6), 220 (7.8), 165 (6.9), 147 (16.4), 120 (100).

Anal. Calcd for $C_{16}H_{20}O_9$: C, 53.93; H, 5.66. Found: C, 53.83; H, 5.98.

Compound 32. To a solution of diisopropylamine (1.7 mL, 12 mmol) in THF (12 mL) at −78 °C was added n-butyllithium (10 mmol, 4.7 mL of 2.12 M in hexane) and stirred for 15 min. Methyl acetate (0.88 mL, 11 mmol) was added, the reaction stirred for 1 h at −78 °C, and 3,4-bis(trimethylsiloxy)benzaldehyde (1.4 g, 5 mmol) added. After 1 h at −78 °C, the reaction was quenched with saturated NH_4Cl (10 mL), warmed to room temperature, and stirred for an additional 6 h. The solution was extracted with EtOAc (3 × 15 mL), and the combined organic layers were dried with Na_2SO_4, and evaporated to give 0.42 g (41%) of **32**: mp 101–102 °C. IR ($CHCl_3$): ν 3700–3100, 1742, 1640, 1528 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.63 (d, J = 6.8 Hz, 2 H), 3.65 (s, 3 H), 4.9 (t, J = 6.9 Hz, 1 H), 6.6–6.8 (m, 3 H), 7.1–7.6 (br s, 3 H). ^{13}C NMR (DMSO-d_6) δ 171.3, 145.0, 144.4, 135.8, 116.6, 115.7, 113.3, 69.3, 51.2, 44.5. MS (70 eV), m/e (relative intensity): 212 (28.7), 194 (44.1), 163 (46.9), 139 (100).

Anal. Calcd for $C_{10}H_{12}O_5$: C, 56.60; H, 5.70. Found: C, 56.70; H, 5.78.

Reaction of L-Ascorbic Acid (1) with 32. To compound **32** (102 mg, 0.5 mmol) in water (5 mL) was added L-ascorbic acid (**1**) (264 mg, 1.5 mmol) and the solution stirred at 50 °C for 3 days. The reaction was evaporated and the residue chromatographed (4:1, EtOAc/hexanes) to afford 64 mg (34%) of bislactone **33** and 76 mg (38%) of lactone ester **34**.

Bislactone **33**: $[\alpha]_D$ −10.3° (c 1.0, 95% EtOH). IR ($CHCl_3$): ν 3600–3100, 1806, 1610, 1529 cm^{-1}. ^{13}C NMR (DMSO-d_6): δ 174.8, 171.3, 145.6, 145.3, 128.3, 123.2, 119.4, 115.8, 105.1, 89.2, 88.5, 74.7, 73.5, 44.3, 33.0. MS (70 eV), m/e (relative intensity): 313 (1.1), 283 (7.3), 255 (94.3), 213 (22.7), 171 (100).

Lactone ester **34**: $[\alpha]_D$ +20.2° (c 1.0, 95% EtOH). IR ($CHCl_3$): ν 3600–3100, 1795, 1768, 1742, 1610, 1540 cm^{-1}. ^{13}C NMR (DMSO-d_6): (open form) δ 210.2, 176.3, 172.8, 145.1, 144.6, 126.7, 120.6, 117.3, 115.0, 80.4, 74.6, 73.3, 60.7, 51.6, 47.2, 32.7; (closed form) δ 174.1, 171.6, 145.1, 144.4, 128.3, 125.0, 119.0, 115.8, 107.8, 86.8, 83.1, 73.9, 69.9, 51.2, 47.6, 31.9. MS (70 eV), m/e (relative intensity): 355 (0.1), 303 (0.1), 284 (0.8), 255 (3.8), 220 (6.8), 194 (91.1), 163 (100).

Leudrin (5). To **33** (102 mg, 0.28 mmol) in THF (3 mL) was added B_2H_6 (0.55 mL, 1 M in THF) and the solution refluxed for 1 h. The reaction was diluted with water (1 mL), evaporated, and chromatographed (3:1, EtOAc/hexanes) to afford 39 mg (38%) of leudrin (**5**): $[\alpha]_D$ −16.7° (c 0.88, 95% EtOH). IR ($CHCl_3$): ν 3650–3100, 1802, 1716, 1640 cm^{-1}. ^{13}C NMR (DMSO-d_6): δ 174.6, 171.9, 145.1, 124.0, 121.7, 119.8, 115.8, 89.9, 79.8, 69.1, 67.9, 61.3, 41.8, 33.4. MS (70 eV), m/e (relative intensity): 340 (17.3), 220 (7.2), 163 (20.3), 136 (100).

Compound 35. Leudrin (**5**) (39 mg, 0.1 mmol) was dissolved in MeOH (0.2 mL) and treated at 0 °C with an ethereal solution of CH_2N_2 until a yellow color persisted. The reaction was stirred over $MgSO_4$ for 1 h, evaporated, and chromatographed (1:1, EtOAc/hexanes) to afford 40 mg (96%) of compound **35**: $[\alpha]_D$ −18.0° (c 1, 95% EtOH) (lit.[30] $[\alpha]_D$ −19.2° (c 0.92, 95% EtOH). IR ($CDCl_3$): ν 3700–3000, 1799, 1522 cm^{-1}. ^{13}C NMR (DMSO-d_6): δ 174.7, 172.0, 147.4, 146.4, 125.9, 121.5, 112.9, 112.0, 89.9, 79.9, 69.1, 68.0, 61.2, 55.6, 41.2, 33.4. MS (70 eV), m/e (relative intensity): 368 (3.0), 354 (33.5), 336 (3.7), 250 (4.3), 177 (22.8), 150 (100).

Acknowledgment. We are indebted to the donors of The Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We thank Professor Chevolet for a sample of delesserine, Professor Perold for leucodrin and dimethyl leudrin, Professor Glombitza for dimethyl methylrhodomelol, and Hoffmann-La Roche for L-ascorbic acid (vitamin C). Funding for the VG-7035 mass spectrometer used in these studies was provided by NSF Instrument Grant CHE-8509862.

Kinetics and Mechanism of the Ketonization of a Conjugated Trienol

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Received September 2, 1987

The steroidal trienol 3-hydroxy-3,5,7-estratrien-17-one (**1**) decomposes in aqueous solution to yield 5,7-estradiene-3,17-dione (**2**), 4,7-estradiene-3,17-dione (**3**), and an unidentified oxidation product (**5**). The relative amounts of the products vary, with **3** predominant at pH ~1 and **2** the major product at pH values between 2 and 8. The oxidation product **5** is formed in significant amounts only in relatively basic solutions (pH >6) and increases as the pH is increased. The rate constant for the overall reaction may be expressed as k^{obsd} (s^{-1}) = (1.24 ± 0.07) × 10^{-2} + (2.54 ± 0.13)[H^+] + (1.78 ± 0.06) × 10^5[OH^-]. The rate constant for protonation of **1** in acidic solution is ca. 200-fold smaller than that for 1-cyclohexenol, presumably due to the extended conjugation of the enol system in **1**. Although the hydroxide-catalyzed rate constant for ketonization of **1** is comparable to the rate constants for conjugated dienols, the uncatalyzed ketonization rate constant for **1** is ca. 10^3-fold slower than that for most dienols. The much slower uncatalyzed rate constant for **1** is due to its inability to undergo the characteristic 1,5-sigmatropic shift of other conjugated enols.

The kinetics and mechanism of the interconversion of simple aldehydes and ketones with their enols has been the subject of active investigation for a long time.[1] Although rate constants for enolization of carbonyl compounds are relatively straightforward to measure by a variety of techniques, it has only recently been possible to monitor this reaction in the thermodynamically favorable direction of enol to aldehyde or ketone. Primarily

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(1) For reviews, see: (a) Bell, R. P. *The Proton in Chemistry*, 2nd ed.; Cornell University Press: Ithaca, NY, 1973. (b) Lamaty, G. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1976; Vol. 2, pp 157–240. (c) Toullec, J. *Adv. Phys. Org. Chem.* **1982**, *18*, 1–77.

0022-3263/88/1953-1540$01.50/0

Table I. Catalytic Constants for the Ketonization of Trienol 1

catalyst	rate constant (M^{-1} s^{-1})[a]
H^+	2.54 ± 0.13
H_2O	(2.25 ± 0.13) × 10^{-4} [b]
OH^-	(1.78 ± 0.06) × 10^5
HOAc	0.100 ± 0.003
OAc^-	1.73 ± 0.03
HPO_4^{2-}	18.0 ± 0.4
$H_2PO_4^-$	2.23 ± 0.11

[a] 25.0 °C, ionic strength = 0.1 (NaCl), 1.7% methanol. Errors are standard deviations. [b] k_0/55 M.

through the work of Kresge[2] and Capon[3] and their coworkers, rate constants are now available for the ketonization of the enols of several simple carbonyl compounds such as acetaldehyde,[2f,3c] acetone,[2b] and acetophenone.[2c] Surprisingly, many of these enols have a lifetime on the order of several seconds to minutes in slightly acidic solutions near room temperature.

Studies on the ketonization of conjugated enols, however, have been less extensive.[4] These compounds are of interest since they are intermediates in the interconversion of β,γ-unsaturated ketones and their α,β-unsaturated isomers.[5] Duhaime and Weedon have generated several dienols in aqueous solution by irradiating the corresponding α,β-unsaturated ketones by using flash photolysis.[4d,e,h] The rate of reketonization of these dienols was monitored in basic solution, enabling values to be measured for the pK_a's of the dienols as well as the rate constants for protonation of the dienolates by water and for ketonization of the neutral dienols. In contrast to simple enols, the neutral dienols examined by Duhaime and Weedon ketonize rapidly, even in neutral solution. The rapid ketonization of these dienols was attributed to an intramolecular 1,5-hydrogen shift to generate the α,β-unsaturated ketones.[4d,e,h] In support of this mechanism, we have recently found that 1,3-cyclohexadienol, a dienol locked in a conformation such that a 1,5-hydrogen shift cannot occur, has a lifetime comparable to that of simple enols in slightly acidic solution.[4g]

In this work we report the results of a kinetic investigation into the ketonization of 3-hydroxy-3,5,7-estratrien-17-one (1). This trienol can be isolated as a solid by protonation of the corresponding enolate ion.[6] Our interest in 1 is due to its unusual stability, as well as the fact that it has been shown to be a substrate for the enzyme 3-oxo-Δ^5-steroid isomerase.[7] Trienol 1 can ketonize three different ways to produce either the $\Delta^{5,7}$ isomer 2, the $\Delta^{4,7}$ isomer 3, or the $\Delta^{4,6}$ isomer 4. Thus, 1 represents the logical intermediate in the interconversions 2 ⇌ 3, 2 ⇌ 4, and 3 ⇌ 4 (eq 1).

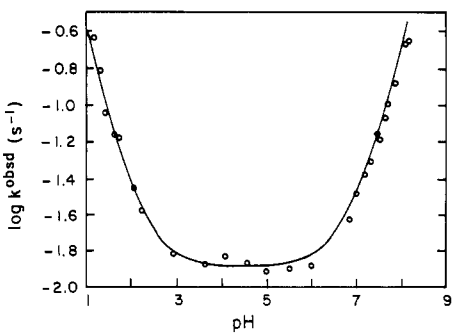
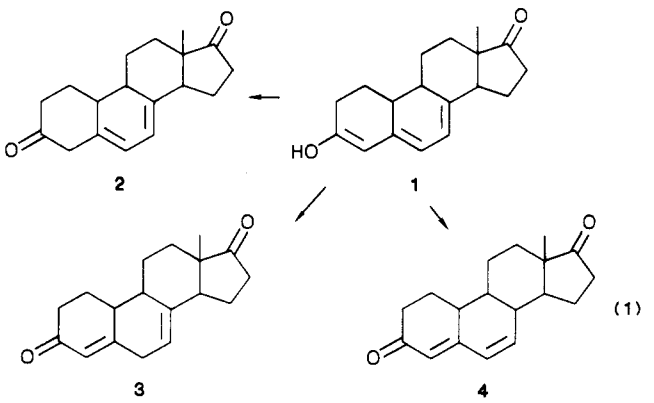


Figure 1. Plot of log k^{obsd} vs pH at [buffer] = 0 for the ketonization of 3-hydroxy-3,5,7-estratrien-17-one (1) at 25.0 °C, μ = 0.1. The curve is theoretical based on eq 1 and using the rate constants given in the text.



Results

Trienol 1 was synthesized by quenching the enolate of 4,6-estradiene-3,17-dione (4) in a mixture of acetic acid–water (1:1).[6] Although 1 is unstable in solution or in contact with air, it can be stored for several days as a solid under vacuum, with only slight decomposition. Because of the instability of 1 in solution, purification was not feasible and the crude product was used in the kinetic experiments. However, the ultraviolet spectrum shows that the trienol is the major product (λ_{max}^{MeOH} 320, ϵ 13 000, lit.[6] 320 nm, ϵ 15 370) and that there is little contamination by 2 (λ_{max}^{MeOH} 281 nm, ϵ 8600),[6] 3 (λ_{max}^{MeOH} 238 nm ϵ 14 950),[6] or 4 (λ_{max}^{MeOH} 280 nm ϵ 26 300).[8] Periodic wavelength scans were routinely taken, and the substrate was discarded when obvious decomposition had occurred.

Kinetics. The ketonization of 1 was monitored at 25.0 °C (μ = 0.1, NaCl) at pH values ranging from 1 to 8 by observing the decay in the UV absorbance at 320 nm. Hydrochloric acid solutions were used to control the pH in the more strongly acidic solutions, whereas acetate, phosphate, and EPPS (*N*-(2-hydroxyethyl)piperazine-*N*'-3-propanesulfonic acid) buffers were used at higher pH's. The pH-dependent rate constants at zero buffer

(2) (a) Chiang, Y.; Kresge, A. J.; Walsh, P. A. *J. Am. Chem. Soc.* **1982**, *104*, 6122. (b) Chiang, Y.; Kresge, A. J.; Tang, Y. S.; Wirz, J. *J. Am. Chem. Soc.* **1984**, *106*, 460. (c) Chiang, Y.; Kresge, A. J.; Wirz, J. *J. Am. Chem. Soc.* **1984**, *106*, 6392. (d) Chiang, Y.; Kresge, A. J.; Walsh, P. A. *J. Am. Chem. Soc.* **1986**, *108*, 6314. (e) Pruszynski, P.; Chiang, Y.; Kresge, A. J.; Schepp, N. P.; Walsh, P. A. *J. Phys. Chem.* **1986**, *90*, 3760. (f) Chiang, Y.; Hojatti, M.; Keeffe, J. R.; Schepp, N. P.; Wirz, J. *J. Am. Chem. Soc.* **1987**, *109*, 4000.

(3) (a) Capon, B.; Rycroft, D. S.; Watson, T. W. *J. Chem. Soc., Chem. Commun.* **1979**, 724. (b) Capon, B.; Rycroft, D. S.; Watson, T. W.; Zucco, C. *J. Am. Chem. Soc.* **1981**, *103*, 1761. (c) Capon, B.; Zucco, C. *J. Am. Chem. Soc.* **1982**, *104*, 7567. (d) Capon, B.; Siddhanta, A. K. *J. Org. Chem.* **1984**, *49*, 255. (e) Capon, B.; Siddhanta, A. K.; Zucco, C. *J. Org. Chem.* **1985**, *50*, 3580.

(4) Dienols have been generated and observed, although their rates of ketonization have rarely been examined. See, for example: (a) Schmidt, E. A.; Hoffmann, H. M. R. *J. Am. Chem. Soc.* **1972**, *94*, 7832. (b) Noyori, R.; Inoue, H.; Kato, M. *J. Am. Chem. Soc.* **1970**, *92*, 6699. (c) Noyori, R.; Inoue, H.; Kato, M. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3673. (d) Duhaime, R. M.; Weedon, A. C. *J. Am. Chem. Soc.* **1985**, *107*, 6723. (e) Duhaime, R. M.; Weedon, A. C. *J. Am. Chem. Soc.* **1987**, *109*, 2479. (f) Turecek, F.; Havlas, Z. *J. Org. Chem.* **1986**, *51*, 4066. (g) Pollack, R. M.; Mack, J. P. G.; Blotny, G. *J. Am. Chem. Soc.* **1987**, *109*, 3138. (h) Duhaime, R. M.; Weedon, A. C. *Can. J. Chem.* **1987**, *65*, 1867.

(5) (a) Ringold, H. J.; Malhotra, S. K. *Tetrahedron Lett.* **1962**, *15*, 699. (b) Malhotra, S. K.; Ringold, H. J. *J. Am. Chem. Soc.* **1963**, *85*, 1538; (c) **1964**, *86*, 1997; (d) **1965**, *87*, 3228. (e) Noyce, D. S.; Evett, M. *J. Org. Chem.* **1972**, *37*, 394; (f) **1972**, *37*, 397. (g) Whalen, D. L.; Weimaster, J. F.; Ross, A. M.; Radhe, R. *J. Am. Chem. Soc.* **1976**, *98*, 7319. (h) Pollack, R. M.; Mack, J. P. G.; Eldin, S. *J. Am. Chem. Soc.* **1987**, *109*, 5048.

(6) Kruger, G. *J. Org. Chem.* **1968**, *33*, 1750.

(7) Bantia, S.; Pollack, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 3145.

(8) Zderic, J. A.; Carpio, H.; Bowers, A.; Djerassi, C. *Steroids* **1963**, *1*, 233.

concentration were obtained as intercepts of buffer plots with acetate (pH 3 to 5.5) and phosphate (pH 5.5 to 7.5) at concentrations of buffer in the range 0.001–0.1 M (acetate) and 0.002–0.02 (phosphate). At high pH values (>7.5), it was impossible to generate buffer plots, as the observed rates in the presence of even moderate amounts of buffer are too high for conventional spectral analysis. Rate constants at these pH's were obtained in very dilute (1×10^{-4} to 2×10^{-4} M) solutions of EPPS. Since the difference in observed rate constants between these buffer solutions is negligible, the measured rates contain no significant contribution from buffer catalysis in these dilute solutions. These low concentrations of buffer are incapable of maintaining good pH control, as pH drops of ca. 0.2 to 0.6 unit were routinely observed upon addition of **1**, presumably due to slight acidic impurities in the trienol. The pH value used under these conditions is the final reading. The catalytic constants for acetate and phosphate were obtained from replots of the observed slope of the buffer plot vs. the mole fraction of the base form of the buffer. These values are given in Table I.

Figure 1 shows the dependence of the buffer-independent rate constant on pH. These results were fit to eq 2, giving values of $k_0 = (1.24 \pm 0.07) \times 10^{-2}$ s^{-1}, $k_H = 2.54$

$$k^{obsd} = k_0 + k_H[H^+] + k_{OH}[OH^-] \qquad (2)$$

$\pm$ 0.13 M^{-1} s^{-1}, and $k_{OH} = (1.78 \pm 0.06) \times 10^5$ M^{-1} s^{-1}. Although the decomposition of the trienol is catalyzed by both acid and base, there is a substantial portion of the pH range where the rate is independent of pH. Catalysis by water is predominant from pH 3.5 to pH 6, whereas proton catalysis becomes significant below pH 3.5 and hydroxide catalysis becomes important above pH 6.

Product Analysis. The products of ketonization of the trienol were analyzed by reverse-phase high performance liquid chromatography, with UV detection at both 238 and 282 nm. Two of the products give retention times that are identical with those for the $\Delta^{5,7}$ and $\Delta^{4,7}$ ketones, **2** and **3**, respectively, whereas the third major product **5** elutes just before the $\Delta^{4,6}$ ketone **4**. Four other products showed up with a small but definite absorbance. These products account for 3–16% of the total peak area in the chromatograms and were not characterized further. In some cases **4** did appear in minor amounts, 1–2% or up to 9% in the cases of high buffer concentration. A possible explanation for this result is the conversion of **3** to **4**, which occurs at high buffer concentrations.

Product **5** was characterized as an oxidation product by mass spectrometry ($M^+ = 286$). The relative amount of **5** decreases when the reaction is run under argon and increases when oxygen is bubbled through the solution prior to addition of the trienol. Complete structure elucidation of **5** was not attempted.

The relative amounts of the three major products were estimated at several pH values between 1 and 8 by measuring the areas of the peaks in the HPLC chromatogram and correcting the observed ratios by the extinction coefficients of the products. The extinction coefficient of **5** was found to be ca. 24000 by simultaneously monitoring a mixture of **4** and **5** by UV detection at 282 nm and refractive index detection. The product ratios under conditions where the reaction is <10% due to buffer catalysis are summarized in Figure 2.

The relative amounts of product ketones were also estimated by an analysis of the ultraviolet spectra after about 10 half-lives. The $\Delta^{4,7}$ ketone **3** could be detected by its absorbance maximum at ca. 246 nm in water, whereas both **2** and **5** show values for λ_{max} in water near 280 nm. A distinction between these latter two compounds was made

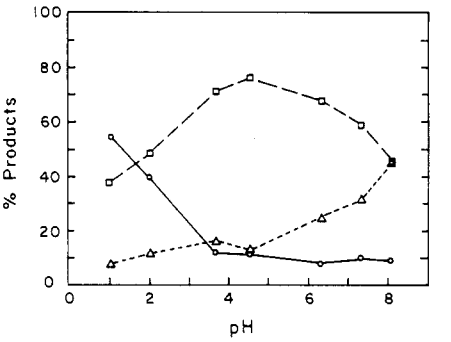


Figure 2. Plot of product composition vs pH at [buffer] = 0 for the reaction of 3-hydroxy-3,5,7-estratrien-17-one (**1**) at 25.0 °C, μ = 0.1: (□) % **2**; (○) % **3**; (△) % **5**.

by the addition of ca. 10^{-10} M 3-oxo-Δ^5-steroid isomerase, which rapidly converts the $\Delta^{5,7}$ isomer to the $\Delta^{4,7}$ isomer, shifting its absorbance maximum from ca. 280 to 248 nm.[7] Residual absorbance at 280 nm then is due to the oxidation product **5**. The loss in absorbance at 280 nm (and increase at 248 nm) is due to **2** originally present in the product of the ketonization reaction. Results in qualitative agreement with the HPLC method were obtained.

Although these analyses are somewhat inexact, it is clear that substantial amounts of **2** are formed at all pH values, whereas **3** is primarily observed in acidic solutions and the oxidation product **5** becomes important only at higher pH's.

Discussion

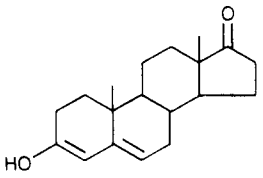
Trienol **1** is unusual in that it can be isolated as a solid from the protonation of the dienolate of **4**. Although kinetically stable enols are not as rare as commonly believed,[9] most have some particular stabilizing feature associated with them such as steric hindrance to ketonization. It is of interest then to determine whether the extended conjugation in **1** causes it to ketonize unusually slowly relative to simple enols or even dienols.

Hydronium Ion Catalyzed Reaction. The acid-catalyzed ketonization of enols involves protonation of the double bond and loss of a proton from the alcohol oxygen. Kresge[2d,f] has argued that the mechanism for this reaction is a two-step one, with carbon protonation preceding conversion of the oxonium ion to the aldehyde or ketone. However, Capon[3c] has suggested that some evidence is best explained by a concerted mechanism. In the absence of evidence to the contrary, we will interpret our results in terms of a simple stepwise mechanism, although there is no compelling evidence on this point for this system.

Protonation of the trienol **1** by hydronium ion can occur at either C-4, C-6, or C-8 (eq 1). Analysis of the products shows that the major reaction is protonation of the neutral trienol at C-6 to give the $\Delta^{4,7}$ isomer **3**, although substantial amounts of **2** are also formed. This result can be compared to the behavior of conjugated dienols in aqueous acid. The dienol **6** has been shown to undergo preferential protonation at C-6 to give the conjugated ketone rather than protonation at C-4 giving the unconjugated isomer.[5d] Similarly, other dienols in which the β-carbon is tertiary protonate more rapidly at the γ-carbon than the α-carbon.[5e] The lack of protonation at C-8 in **1** to produce the

(9) For examples of stable simple enols, see: (a) Hart, H. *Chem. Rev.* **1979**, *79*, 515. (b) Biali, S. E.; Rappoport, Z.; Hull, W. E. *J. Am. Chem. Soc.* **1985**, *107*, 5450. (c) Nugiel, D. A.; Rappoport, Z. *J. Am. Chem. Soc.* **1985**, *107*, 3669. (d) Kaftory, M.; Biali, S. E.; Rappoport, Z. *J. Am. Chem. Soc.* **1985**, *107*, 1701. (e) Biali, S. E.; Rappoport, Z. *J. Am. Chem. Soc.* **1985**, *107*, 1007. (f) Nadler, E. B.; Rappoport, Z. *J. Am. Chem. Soc.* **1987**, *109*, 2112. (g) Rappoport, Z. *J. Am. Chem. Soc.* **1987**, *109*, 4730.

fully conjugated ketone may be attributed to steric hindrance at that position. Rogers and Sattar[10] have shown that in the analogous protonation reactions of dienol ethers, substitution at the γ-position by an alkyl group substantially retards protonation at that position relative to C-α. For example, compound **7** protonates preferentially (95%) at C-γ in acid, whereas **8** reacts primarily (88%) at C-α.



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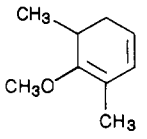

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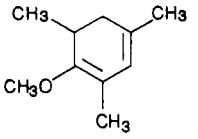


8

The rate constant for protonation of **1** in acid cannot be compared to the analogous rate constant for other dienols, as no other rate constants for acid-catalyzed ketonization of dienols have been reported. However, a comparison with rate constants for a simple enol with similar steric interactions is instructive. The rate constant for protonation of 1-cyclohexenol can be obtained from the rate constant for acid-catalyzed enolization[11a] of cyclohexanone and the keto–enol equilibrium constant.[11b] This value (590 M^{-1} s^{-1}) is about 200-fold larger than the sum of the rate constants for protonation of **1** at C-4 (k_α) and C-6 (k_γ). The lower value of k_α compared to cyclohexenol is probably due to the loss of conjugation of the double bonds in the B ring upon protonation. Similarly, protonation at C-6 may be retarded by the loss of conjugation of the 7,8 double bond.

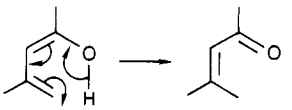
Hydroxide-Catalyzed Reaction. Hydroxide ion catalyzed ketonization of enols proceeds via ionization of the enol followed by protonation of the enolate ion.[2,3] In analogy with the acid-catalyzed reaction, protonation of the enolate may occur at C-4, C-6, or C-8 to give **2**, **3**, or **4** directly. HPLC analysis of the reaction products at very low buffer concentrations and pH 6 to 8 shows that the predominant product is, in fact, none of these but rather the unidentified oxidation product **5**. However, of the possible products due to ketonization, the one formed from protonation of the anion at the α-carbon, **2**, is the most favored. Again, as in the acid-catalyzed reaction, there appears to be little, or no, protonation at C-8, presumably due to steric hindrance at that position.

Positions of protonation of conjugated enolate ions have been determined for several dienolates. Whalen et al.[5g] have found that the dienolate ion formed from 3-cyclohexenone is protonated 575 times faster at C-α than at C-γ by $D_2PO_4^-$ in D_2O. The same ratio for the dienolate of 3-cyclopentenone is 3.2.[5g] The anion from 5-androstene-3,17-dione also protonates more rapidly at C-α than at C-γ (k_α/k_γ = 20).[5h] Thus, the position of protonation of the trienolate ion by water parallels the protonation of the corresponding dienolate ion.

The hydroxide ion catalyzed rate constant (1.78 × 10^5 M^{-1} s^{-1}) may be compared with rate constants previously measured for the ketonization of other enols in base. Rate constants for the reaction of a series of conjugated dienols have been measured by Duhaime and Weedon.[4d,e] They found that the rate constant for protonation of the dienolate ion (k_β) by water is ca. 10^3 s^{-1} for several dienolates. The pK_a's of these dienols are all between 10.4 and 12.0, so that the second-order rate constant ($k_{OH} = k_\beta K_a/K_w$) at pH < pK_a ranges between 2.7 × 10^4 s^{-1} and 1.3 × 10^6 s^{-1}. Although Duhaime and Weedon were able to separate these two factors in their systems, we have not been able to determine the pK_a of **1**. However, it is clear that the overall rate constants (k_{OH}) are comparable in all the conjugated systems investigated including **1**. In all cases, the rates of reaction with hydroxide ion are in the range of 10^4 to 10^6 M^{-1} s^{-1}.

Hydroxide-catalyzed ketonization rates of a variety of simple enols have also been measured. These rate constants depend markedly on the substitution pattern at the enolate carbon being protonated by water. For the enols of acetone[2b] and acetaldehyde,[2f] k_{OH} at 25 °C is 3.4 × 10^7 M^{-1} s^{-1} and 1.7 × 10^6 M^{-1} s^{-1}, respectively, although k_{OH} is reported to be 1.50 × 10^7 M^{-1} s^{-1} at 15 °C[3c] for the enol of acetaldehyde. Similarly for 3-chloro-1-propen-2-ol,[3e] k_{OH} = 4.7 × 10^7 M^{-1} s^{-1} (15 °C). Substitution at the methylene carbon decreases the rate significantly. Thus k_{OH} for (*Z*)-1-propen-1-ol and 2-methyl-1-propen-1-ol is 5.0 × 10^4 M^{-1} s^{-1} (15 °C) and 1.4 × 10^3 M^{-1} s^{-1} (15 °C), respectively.[3e] α-Carbon substitution appears to have little effect on the rate constant. The observed rate constant for reaction of **1** with hydroxide ion is due primarily to protonation at a monosubstituted methylene carbon atom. The rate constant for this process is similar to that for 2-methyl-1-propen-1-ol, a simple enol with a monosubstituted methylene.

Neutral Reaction. The simplest mechanism for the pH-independent ketonization of enols is ionization to the enolate ion followed by protonation with acid,[2c,f] although a concerted mechanism has been considered.[3c,e,13] The rate constant for **1** is substantially lower than the uncatalyzed rate constants for all dienols that have been investigated (ca. 10 s^{-1}),[4d,e] except for 1,3-cyclohexadienol (1.2 × 10^{-2} s^{-1}).[4g] The rapid ketonization rates for these dienols have been attributed to a 1,5-sigmatropic hydrogen shift that leads to the conjugated ketone (eq 3).[4d,e,h] This process



(3)

is not available to either **1** or 1,3-cyclohexadienol since the two C=C double bonds in these enols are locked in a conformation that does not allow the 1,5-hydrogen shift to occur. Further confirmation of the difference in mechanism between the rapidly reacting and slowly reacting conjugated enols may be seen in the fact the water-catalyzed reaction for both **1** and 1,3-cyclohexadienol gives the unconjugated isomer, whereas for the other dienols, the conjugated isomer is the major product. The inability of both **1** and 1,3-cyclohexadienol to undergo a 1,5-hydrogen shift is no doubt a major reason for their relatively slow rates of ketonization.

Experimental Section

Materials. 3-Hydroxy-3,5,7-estratrien-17-one (**1**) was synthesized by quenching the enolate of 4,6-estradiene-3,17-dione (**4**) in acetic acid/water (1:1).[6] Compound **4** was obtained either by synthesis according to Zderic et al.[8] or commercially (Steraloids). The trienol **1** is moisture-sensitive and must be kept under vacuum. Exposure to water vapor in the air can decompose the trienol within a few hours, although the trienol will stay pure under vacuum for several weeks. For these studies the trienol was used within a week of synthesis, λ_{max}^{MeOH} 320, ε 13 000, lit.[6] 320 nm, ε

(10) Rogers, N. A. J.; Sattar, A. *Tetrahedron Lett.* **1965**, 1471.
(11) (a) Dubois, J.-E.; El-Alaoui, M.; Toullec, J. *J. Am. Chem. Soc.* **1981**, *103*, 5393. (b) Toullec, J. *Tetrahedron Lett.* **1984**, *25*, 4401.
(12) Kresge, A. J.; Sagatys, D. S.; Chen, H. L. *J. Am. Chem. Soc.* **1977**, *99*, 7228.
(13) Tapuhi, E.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 5758.

15 700. Water was double distilled and all other reagents were reagent grade.

Kinetic Procedure. The decomposition of **1** was monitored at 320 nm in a Gilford Response spectrophotometer. Immediately before use, a small sample of **1** was removed from under vacuum and dissolved in 50 μL of methanol. With use of a hollow glass stirring rod that was bent up at one end, this solution was added to a cuvette containing temperature-equilibrated buffer solution. For rapid reactions, the spectrophotometer was equipped with a small hole in the sample compartment with a removable rubber stopper directly above the cuvette. The stopper was removed and the trienol was added and stirred (using the glass rod device) as quickly as possible and the stopper was replaced. With practice, this procedure could be completed in a few seconds.

The decomposition of **1** showed excellent pseudo-first-order kinetics with stable infinity points. The data were analyzed by fitting the points to an exponential curve using a nonlinear least-squares computer program. Plots of observed rate constants vs buffer concentration were extrapolated to zero buffer to get the buffer-independent rate constants. The pH values of the solutions generally did not change significantly when measured before and after a run. However, in the case of the dilute EPPS buffers used to determine the hydroxide ion catalyzed rate constants, there was a large (ca. 0.2 to 0.6 unit) pH drop upon addition of **1**. The pH used in the calculations was the final pH. pH readings for various concentrations of the other buffers were all within ±0.03 of each other at the same nominal pH. If necessary, small amounts of acid or base were added to adjust the pH of the buffer solutions before use.

Product Studies. HPLC Method. A trace amount of **1** was taken from under vacuum and dissolved in 200 μL of methanol. This solution was added to 3 mL of a dilute buffer solution of the appropriate pH. The concentration of **1** in the final solution was approximately 10^{-4} M. The trienol was allowed to react for approximately 5 half-lives. The steroids were extracted with 1 mL of methylene chloride. The methylene chloride was dried with a small amount of magnesium sulfate and the magnesium sulfate was filtered off. Fifteen microliters of the filtrate were injected into the HPLC. The solution was injected immediately, as the steroids may further isomerize to other products. The buffer concentrations were from 1×10^{-5} M to 5×10^{-5} M to ensure that the contribution to the rate of decomposition by the buffer is negligible. A blank was run by using the same procedure as above but without **1**.

The steroids were analyzed on a Waters μ-Porocil 3.9 mm × 30 cm silica gel column with 10% isopropyl alcohol in hexanes. Two UV detectors were used in series to allow simultaneous monitoring of the absorbance at 238 and 282 nm. Relative amounts of products were calculated by using the known extinction coefficients of **2** and **3**. The extinction coefficient of the oxidation product **5** was determined by running the reaction at a higher concentration of **1** and simultaneously monitoring the HPLC eluent by refractive index detection and by UV with added **4** as a standard. Corrections due to peaks in blank runs were made when necessary.

UV Method. Wavelength scans were performed from 220 to 400 nm. Buffer concentrations of 1×10^{-4} M were used. A trace amount of **1** was dissolved in 50 μL of methanol and added to 3 mL of buffer solution. After 5 half-lives, the spectrum was scanned and the absorbance ratio at 280 and 238 nm recorded. A sufficient amount of the enzyme 3-oxo-Δ^5-steroid isomerase was added to cause complete isomerization of **2** to **3** within 1 min, with a concomitant loss of absorbance at 280 nm and an increase at 238 nm. At high pH (around 8) and low pH (around 1 to 2), **2** is spontaneously converted into **3**. Furthermore, at pH 2.5 and below the enzyme is inactive. Under these conditions the reaction was run until the trienol had completely reacted. One milliliter was taken from the solution and added to 3 mL of 0.1 M sodium acetate to provide a suitable pH for enzyme activity as well as a pH where **2** does not convert to **3** to any significant extent. The conversion of **2** to **3** under the reaction conditions is not as fast as the decomposition of trienol so that when the solution was neutralized little interconversion had occurred. Isomerase was added to the acetate buffer and analysis proceeded as before.

Acknowledgment. This work was supported by Grant No. GM 33059 and GM 38155 from the National Institutes of Health.