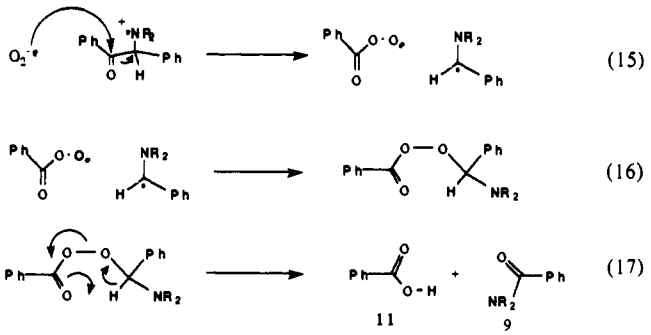
rangement as shown in eqs 15-17. It seems reasonable then that



(15)

(16)

(17)

11 9

the major products produced from the SET quenching in benzene arise from subsequent "cooperative" reactions of the oppositely charged ion-radicals produced within the contact ion pair. The origin of the remaining products is less clear. While benzaldehyde, deoxybenzoin, and benzil are all formed in the direct photolysis of **7**, it seems unlikely that their production in the reaction sensitized via singlet oxygen is possible since no light directly absorbed by **7** was used and we were unable to detect any dehydromorpholine, the expected byproduct of a type II photoelimination reaction.[40,41] We suggest benzaldehyde likely arises from unassisted fragmentation of the amino ketone cation radical; indeed this pathway has been detected in earlier studies of amino ketone reactions through reaction with photoexcited acceptors.[42] The origin of **10** seems most likely to be via reduction of amino ketone **7** by its scavenging of a radical intermediate (such as those generated in eqs 13 and 15) and subsequent reactions of the radical so generated. We have recently observed cases where amino ketones such as **7** give **10** or structurally similar reductive deamination products where the primary excited-state quenching process should be SET oxidation of the amino ketone;[10] activation of amino ketones such as **7** by SET from excited donors leads to **10** as the major product in a relatively clean reaction. The origin of the remaining minor product formylmorpholine is unclear; although it is observed in each case where the reaction is sensitized by RB or RBD, it is not detected when PdTPP is used as a sensitizer, and thus it is possible that it does not arise directly from reaction of **7** but possibly as a product from reaction of morpholine liberated in the photoreaction.

Acknowledgment. We thank the U.S. Department of Energy (Grant Number DE-FG02-86ER13504) for support of this research.

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Generation and Stability of a Simple Thiol Ester Enolate in Aqueous Solution

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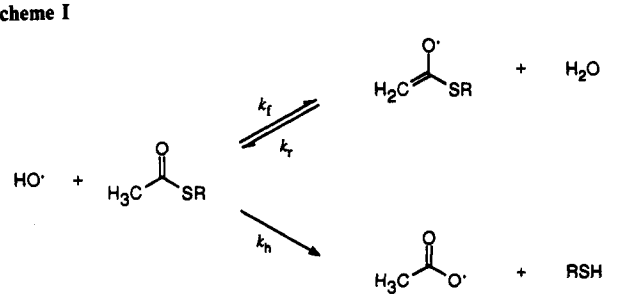
Abstract: The exchange for deuterium of the α-protons of ethyl thioacetate and of acetone in 3-quinuclidinone buffers in D_2O at 25 °C and pD = 7.7-9.3 was followed by ^1H NMR spectroscopy. The exchange reactions lead to the appearance of signals due to the α-CH_2D and α-CHD_2 species that are cleanly resolved from each other and from the signal due to the α-CH_3 species. Observed rate constants for the 3-quinuclidinone-catalyzed exchange were determined during exchange of 30-37% of the first α-proton of each methyl group of ethyl thioacetate or acetone. The rate constants for exchange correspond to those for deprotonation of ethyl thioacetate and acetone by 3-quinuclidinone to give the free enolates, with $k_B = 2.2 \times 10^{-5}$ and 5.2×10^{-4} M^{-1} s^{-1}, respectively. These rate constants were combined with the known pK_a of acetone to estimate $pK_a = 20.4$-21.5 for ethyl thioacetate and $k_{BH} = 1.7 \times 10^8$ to 2×10^9 M^{-1} s^{-1} for the reaction of the free thiol ester enolate with the 3-quinuclidinone cation. The lifetime of the buffer acid-enolate intimate ion pair $BH^+ \cdot {}^-CH_2COSEt$ with respect to proton transfer to give B-CH_3COSEt is estimated to be from 10^{-9} to 10^{-10} s. These results provide evidence against the suggestion that enzyme-catalyzed Claisen condensation and related reactions proceed by concerted mechanisms that are enforced by the insignificant lifetime of the thiol ester enolate in the presence of an acidic amino acid residue at the enzyme.

The enolates of simple[1] thiol esters of coenzyme A are putative intermediates of numerous important enzymatic reactions such as Claisen-type condensation and the dehydration of β-hydroxy thiol esters.[2-5] However, it is not known whether simple thiol ester enolates are long-lived enough to be formed as enzyme-bound intermediates,[6] and there is scant evidence for the formation of

Scheme I



(1) In this paper, a "simple" thiol ester refers to a thiol ester in which the carbonyl group is adjacent to a carbon atom bearing no other electron-withdrawing groups.
(2) (a) Gehring, U.; Lynen, F. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1972; Vol. 7, Chapter 13. (b) Higgins, M. J. P.; Kornblatt, J. A.; Rudney, H. Reference 2a, Chapter 14. (c) Spector, L. B. Reference 2a, Chapter 12.
(3) (a) Bruice, T. C.; Benkovic, S. J. *Bioorganic Mechanisms*; W. A. Benjamin, Inc.: New York, 1966; Vol. 1, Chapter 3. (b) Kluger, R. *Chem. Rev.* **1990**, *90*, 1151-1169.
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these carbanions in aqueous solution.[7] This has led to the questions of whether enzyme-catalyzed Claisen condensation and related reactions follow concerted pathways that avoid the formation of highly unstable thiol ester enolates[5,6,8] and whether such concerted pathways are *enforced* by the insignificant lifetime of these carbanions in the presence of acidic functional groups present in an enzyme active site.[6]

There are no reliable data pertaining to the effect of a thiol substituent on the acidity of simple α-carbonyl carbon acids. In principle, the pK_a of a thiol ester in aqueous solution could be determined by measuring rate constants for its hydroxide-catalyzed formation (k_f, Scheme I) and breakdown by reaction with water (k_r, Scheme I). However, the determination of k_f is severely hampered by competing base-catalyzed hydrolysis to give acetate ion (k_h, Scheme I). In a previous report,[9] the exchange of tritium from ^3H_2O into ethyl thioacetate catalyzed by 0.17–0.34 M hydroxide ion was accompanied by fast competing hydrolysis to give acetic acid, which had a specific radioactivity of only ca. 0.5% that of the ^3H_2O. However, the decreases in the rates of hydroxide-catalyzed hydrolysis and of aminolysis by tertiary amines of simple thiol esters with decreasing pH or pK_a of the amine nucleophile, respectively, should be greater than the decrease in the rate of tertiary amine-catalyzed deprotonation of a thiol ester, so that the latter reaction will be increasingly favored as the pH and the pK_a of the amine catalyst are lowered.[10] This analysis suggests that the use of a tertiary amine catalyst at neutral pH in D_2O would avoid the problems of both hydrolysis and an unfavorable discrimination isotope effect,[13] so that deuterium exchange into the α-position of a thiol ester could be readily followed by ^1H NMR spectroscopy.

These predictions are confirmed in the present report of the generation of a simple thiol ester enolate as an intermediate in 3-quinuclidinone-catalyzed exchange for deuterium of the α-protons of ethyl thioacetate in D_2O. We have also generated the enolate of acetone in the same way, and comparison of the rate constants for deprotonation of the two carbon acids by 3-quinuclidinone allows us to make a very good estimate of the pK_a of ethyl thioacetate.

Experimental Section

Materials. Ethyl thioacetate was from Lancaster. Spectrophotometric grade acetone, 3-quinuclidinone hydrochloride, potassium deuteroxide (40% wt, 98+% D), and deuteriated methanol (99.8% D) were from Aldrich. Deuterium oxide (99.9% D), deuterium chloride (35% w/w, 99.5% D), and deuteriated chloroform (99.96% or 99.8% D) were from Cambridge Isotope Laboratories. 3-Quinuclidinone hydrochloride was recrystallized from ethanol/water. All other chemicals were reagent grade and were used without further purification.

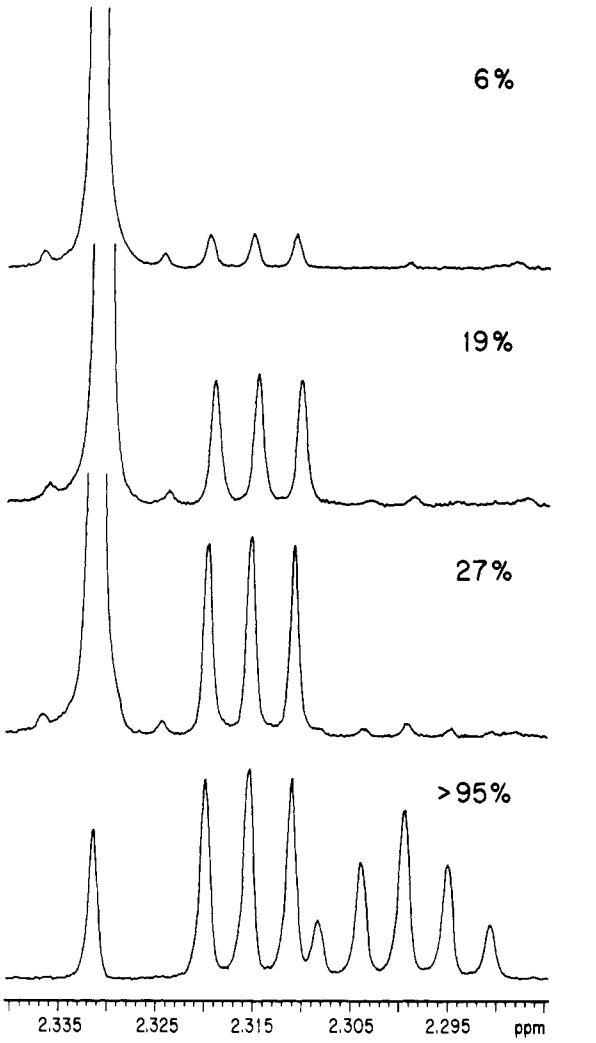

Figure 1. Partial 500-MHz ^1H NMR spectra (recorded in $CDCl_3$) of recovered ethyl thioacetate obtained during exchange of the α-protons for deuterium in the presence of 3-quinuclidinone buffers in D_2O at 25 °C and pD = 7.7–9.3. The tiny peaks on either side of the large singlet at 2.331 ppm in the top three spectra are ^{13}C satellites arising from coupling of the α-CH_3 protons to the neighboring carbonyl carbon. The other peak assignments are given in the text. The fraction of exchanged material, which corresponds to the fraction of exchange of the first α-proton, is indicated at the top right of each spectrum.

Preparation of Solutions. Solutions of potassium deuteroxide and deuterium chloride were prepared by dilution of 40% wt and 35% w/w solutions, respectively, with D_2O. The acidic proton of 3-quinuclidinone hydrochloride was exchanged by dissolving the hydrochloride in D_2O, followed by removal of the solvent by evaporation under reduced pressure. This was repeated twice more, followed by drying in vacuo at 50 °C overnight. Buffers were prepared by dissolving deuteriated 3-quinuclidinone hydrochloride and KCl in D_2O, followed by the addition of an appropriate amount of 2.7 M KOD to give solutions of buffer at various acid/base ratios and I = 1.0 (KCl). Values of pD were obtained by adding 0.4 to the observed pH meter reading.[15]

Kinetic Methods. Rate constants for the 3-quinuclidinone-catalyzed exchange of the α-protons of ethyl thioacetate and acetone in D_2O at 25 °C and ionic strength 1.0 (KCl) were determined by following the disappearance of the substrate and the appearance of the deuteriated products by ^1H NMR spectroscopy. Reactions in a volume of 8 mL were initiated at zero time by injecting 20 μL of a solution of ethyl thioacetate in CD_3OD or 16 μL of neat acetone into the reaction mixture and vortexing, giving final substrate concentrations of ca. 5 and 28 mM, respectively. At various times, an aliquot (1 mL) was withdrawn and quenched with 1.8 M DCl (0.5–0.9 mL). Ethyl thioacetate or acetone was extracted by adding 1.2 mL of $CDCl_3$, followed by vortexing and removal of the aqueous layer with a Pasteur pipet. The organic layer was

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(10) The rate-limiting step for aminolysis by tertiary amines of simple thiol esters is expected to be the expulsion of the relatively basic thiol anion leaving group from the tetrahedral addition intermediate. The values of $\beta_{nuc} \approx 1.0$ for related aminolysis reactions in which expulsion of the leaving group is also rate-limiting[11] and the pH dependence of hydroxide-catalyzed hydrolysis are larger than the values of β = 0.5–0.9 for base-catalyzed deprotonation of simple α-carbonyl compounds,[12] so the latter reaction should be increasingly favored on moving to more weakly basic catalysts and lower [HO^-].
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(13) The discrimination isotope effect for detritiation of acetophenone is k_H/k_T = 15,[14] but there can be no discrimination between heavy and light isotopes in 100% D_2O.
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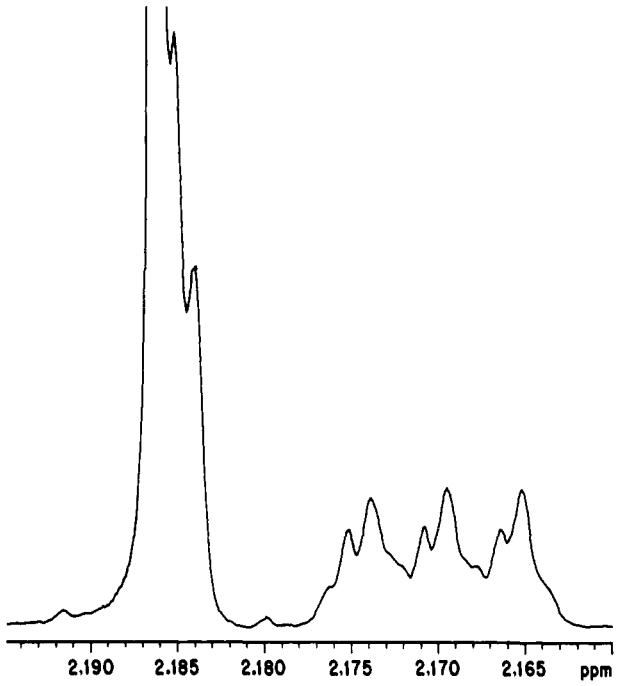


Figure 2. Partial 500-MHz ^1H NMR spectrum (recorded in $CDCl_3$) of recovered acetone obtained after exchange of ca. 37% of the first α-proton of each methyl group[16] (ca. 12% of all protons) for deuterium in the presence of 3-quinuclidinone buffer in D_2O at 25 °C and pD = 8.3. The tiny peaks on either side of the signal at 2.186 ppm are ^{13}C satellites arising from coupling of the α-CH_3 protons to the neighboring carbonyl carbon. The other peak assignments are given in the text.

dried by filtration through a short column of $MgSO_4$ directly into an NMR tube.

^1H NMR Spectroscopy. ^1H NMR spectra at 500 MHz were recorded in $CDCl_3$ on a Varian VXR-500S spectrometer. Values of T_1 for the α-CH_3 and α-CH_2D protons of ethyl thioacetate (spectra run at 25 °C) and acetone (spectra run at 10 °C) were determined to be ca. 5 and 6 s, respectively. Spectra were recorded with a sweep width of 4000 Hz, a 90° pulse angle, an acquisition time of 6–8 s, and zero-filling of the data to 128 000 data points. In all cases, the relaxation delay between pulses was at least 10-fold greater than the longest T_1. The spectra were referenced to $CHCl_3$ at 7.27 ppm, and base lines were drift-corrected before integration of the signals due to the α-CH_3 and α-CH_2D groups.

Results

Figure 1 shows partial 500-MHz ^1H NMR spectra of recovered ethyl thioacetate obtained during exchange of the α-protons for deuterium in the presence of 3-quinuclidinone buffers in D_2O at 25 °C and pD = 7.7–9.3. At early times, the singlet at 2.331 ppm due to the α-CH_3 group is replaced by an upfield triplet at 2.315 ppm (J = 2.2 Hz), which at later times is replaced by a quintet further upfield at 2.299 ppm (J = 2.2 Hz); the latter signals are due to the α-CH_2D and α-CHD_2 groups of mono- and dideuteriated ethyl thioacetate, respectively.

Figure 2 shows the partial 500-MHz ^1H NMR spectrum of recovered acetone obtained after exchange of ca. 37% of the first α-proton of each methyl group[16] (ca. 12% of all protons) for deuterium in the presence of 3-quinuclidinone buffer in D_2O at 25 °C and pD = 8.3. In this case, the disappearance of the singlet at 2.186 ppm due to the two equivalent α-CH_3 groups of acetone is accompanied by the appearance of an upfield triplet at 2.170 ppm (J = 2.2 Hz) due to the α-CH_2D group of monodeuteriated acetone. This triplet is further split by long-range coupling (J = 0.6 Hz) to the remaining α-CH_3 group.[17] The triplet due to the remaining α-CH_3 group of monodeuteriated acetone at 2.185 ppm (J = 0.6 Hz) is only slightly upfield (0.001 ppm) of the signal due to undeuteriated acetone and is therefore only partially resolved.

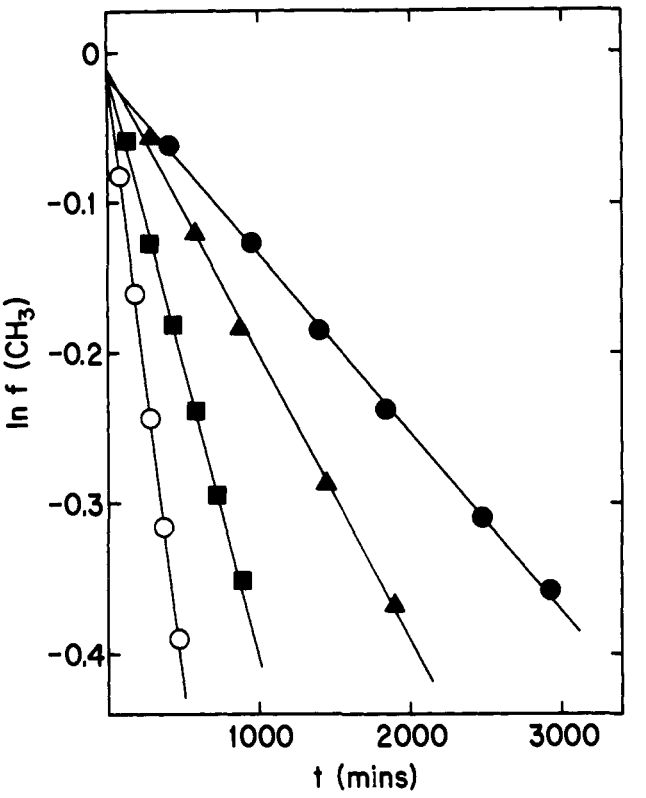
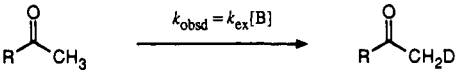


Figure 3. Representative logarithmic plots of the fraction of the remaining α-CH_3 groups, $f(CH_3)$ (see text), against time for the exchange for deuterium of the first proton of the individual α-CH_3 groups of ethyl thioacetate and acetone in 3-quinuclidinone buffers in D_2O at 25 °C and I = 1.0 (KCl): (●) ethyl thioacetate in 0.40 M 3-quinuclidinone buffer, 20% free base; (▲), ethyl thioacetate in 0.25 M 3-quinuclidinone buffer, 50% free base; (■) ethyl thioacetate in 0.30 M 3-quinuclidinone buffer, 90% free base; (○) acetone in 0.10 M 3-quinuclidinone buffer, 50% free base.

Scheme II



R = EtS, Me

The kinetics of deuterium exchange were followed by monitoring the integrated areas of the signals due to the α-CH_3 and α-CH_2D groups during exchange of ca. 30% and 37% of the first α-proton of each methyl group (which corresponds to ca. 10% and 12% of the total α-protons) of ethyl thioacetate and acetone, respectively.[18] During this time, there was no detectable formation of compounds containing α-CHD_2 groups.

After exchange of >95% of the first α-proton of ethyl thioacetate, integration of the NMR signals due to the methylene groups of ethanethiol and ethyl thioacetate showed that there was <8% hydrolysis of the total ethyl thioacetate to give acetate and ethanethiol, so hydrolysis accompanying the exchange of 30% of the first α-proton (the portion of the reaction used to determine the rate constant for exchange) is negligible.

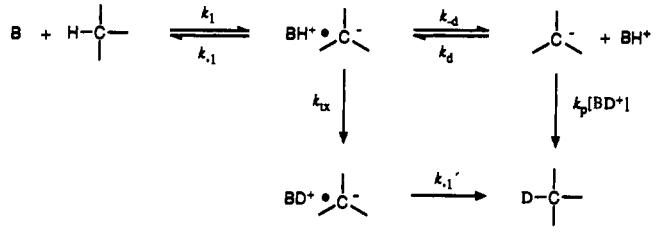
Pseudo-first-order rate constants $k_{obsd} = k_{ex}[B]$ (B = 3-quinuclidinone) for the exchange of the first α-proton of the individual methyl groups of ethyl thioacetate and acetone were

(16) The exchange of the first α-proton of each methyl group of acetone refers to the exchange of either the first proton of acetone, to give $H_2DCCOCH_3$, or the first proton of the remaining methyl group of the latter compound, to give $H_2DCCOCH_2D$.

(17) The coupling pattern cannot be analyzed in detail because the signal due to the α-CH_2D group of monodeuteriated acetone is complicated by the presence of an unknown quantity of $H_2DCCOCH_2D$ in which there is no long-range coupling.

(18) We make the assumption that the rate constant for exchange of the first proton of the residual methyl group of $H_2DCCOCH_3$ is unaffected by the remote deuterium substituent and is the same as that for exchange of the first proton of a single methyl group of acetone.

Scheme III



obtained from the slopes of linear plots of reaction progress against time, according to eq 1 derived for Scheme II.[19] In eq 1, $f(CH_3)$

$$\ln f(CH_3) = -k_{obsd}t \quad (1)$$

$$f(CH_3) = A(\alpha\text{-}CH_3)/[A(\alpha\text{-}CH_3) + 1.5A(\alpha\text{-}CH_2D)] \quad (2)$$

is the fraction of unexchanged methyl groups remaining, calculated from the integrated areas (A) of the peaks due to the α-CH_3 and the α-CH_2D groups according to eq 2. Figure 3 shows representative data for both ethyl thioacetate and acetone plotted according to eq 1.

Discussion

Deuterium Exchange Monitored by ^{1}H NMR Spectroscopy. Figures 1 and 2 show that the exchange for deuterium of the individual α-protons of ethyl thioacetate and acetone can be monitored by 500-MHz ^{1}H NMR spectroscopy because there is very good resolution of the signals due to the undeuteriated methyl groups of the substrates and the monodeuteriated and dideuteriated methyl groups of the products. The upfield isotope shifts (0.016 ppm/D) and H–D coupling constants are similar to those observed in other systems.[20]

The exchange of only the first α-proton of each methyl group of ethyl thioacetate or acetone[16] was followed by studying the reaction over a time period in which there is no detectable formation of products that contain α-CHD_2, and hence α-CD_3, groups. This circumvents the need for an internal standard, because the fraction of exchange can be obtained by directly comparing the integrated areas of the signals due to the α-CH_3 and α-CH_2D groups according to eq 2 rather than monitoring the decrease in the normalized integrated areas of signals due to the substrate.

To the best of our knowledge, this work represents the first use of the ^{2}H perturbation of ^{1}H chemical shifts to follow the exchange reactions of heavy isotopes at carbon. The direct analysis of exchange by this method is simpler and considerably faster than following deuterium exchange by mass spectroscopy[21] or following the exchange of tracer levels of tritium,[9] because for these methods the substrate must be purified for each analysis. The washout of tritium from highly tritium-enriched substrates has been monitored by ^{3}H NMR,[22] but these experiments are subject to the rather severe problems associated with the use of tritium with very high specific radioactivities.

Rate Constants and Mechanism for Deuterium Exchange. Figure 4 shows that when the observed rate constants k_{obsd} for exchange for deuterium of the first α-proton of ethyl thioacetate determined at three different buffer base/acid ([B]/[BD$^+$]) ratios are plotted against the concentration of the basic form of 3-quinuclidinone, the data are correlated by a single line of slope $k_{ex} = 2.2 \times 10^{-5}$ M^{-1} s^{-1}. This constant slope with changing [DO$^-$] and [BD$^+$] shows that catalysis of exchange by the 3-quinuclidinone cation and/or deuteroxide ion is negligible under these conditions and that there is no third-order term corresponding to concerted formation of the enol.[23] Essentially all of the exchange reaction results from general base catalysis by 3-quinuclidinone.

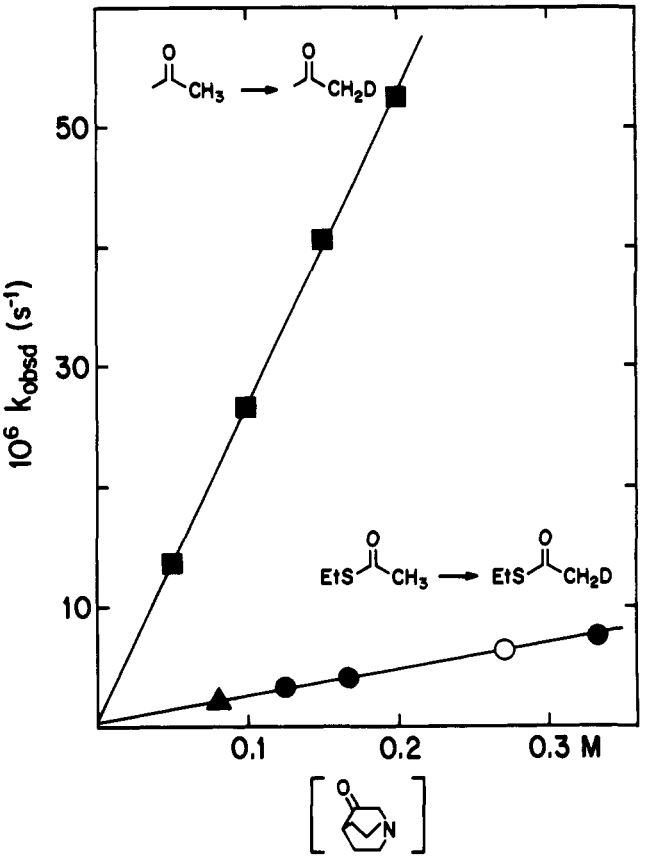


Figure 4. Plot of observed rate constants for exchange for deuterium of the first α-proton of each methyl group of ethyl thioacetate and acetone in 3-quinuclidinone buffers in D_2O at 25 °C and I = 1.0 (KCl) against the concentration of the basic form of 3-quinuclidinone: (■) acetone at [B]/[BD$^+$] = 1, pD = 8.3; (▲) ethyl thioacetate at [B]/[BD$^+$] = 0.25, pD = 7.7; (●) ethyl thioacetate at [B]/[BD$^+$] = 1, pD = 8.3 (○) ethyl thioacetate at [B]/[BD$^+$] = 9, pD = 9.3.

Similarly, the values of k_{obsd} for exchange of the first α-proton of the individual methyl groups of acetone at [B]/[BD$^+$] = 1 (Figure 4) are correlated with a slope $k_{ex} = 2.6 \times 10^{-4}$ M^{-1} s^{-1} for general base catalysis by 3-quinuclidinone.

Possible mechanisms for 3-quinuclidinone-catalyzed exchange for deuterium of the α-protons of ethyl thioacetate and acetone in D_2O are shown in Scheme III. The encounter of the buffer base and proton abstraction (k_1) gives a buffer acid–carbanion intimate ion pair which may then undergo either diffusional separation (k_{-d}) to give the free carbanion or reprotonation by BH$^+$ (k_{-1}) to regenerate the protonated substrate. Once formed, the free carbanion can be protonated only by a molecule of deuteriated buffer acid (k_p[BD$^+$]), resulting in an exchange event, because in D_2O the concentration of the protonated buffer acid BH$^+$ is negligible. The following observations show that the deuterium exchange reactions of ethyl thioacetate and acetone result from reaction of the *free* enolates with BD$^+$.

(1) The exchange reaction does not arise from exchange (k_{tx}) within the intimate ion pair BH$^+$·$^-$$CH_2COR$, because the rate constants for exchange of the proton of BH$^+$ for deuterium from solvent and/or a second molecule of BD$^+$ are considerably smaller

(19) The quantity k_{obsd} in eq 1 and Scheme II refers to the observed rate constant for the disappearance of the individual methyl groups of ethyl thioacetate or of acetone. In the case of acetone, this will be equal to half the rate of disappearance of acetone itself, because exchange of the first proton of acetone, to give $H_2DCCOCH_3$, constitutes the disappearance of acetone but only half of the methyl groups have been consumed.

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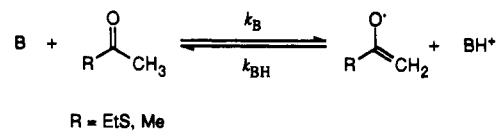
Scheme IV



than that for diffusional separation of the ion pair to give the free enolate ($k_{-d} \gg k_{tx}$).[24]

(2) The protonation of the carbanion within the intimate ion pair $BH^{+}\cdot^{-}CH_2COR$ by a molecule of deuteriated solvent can also be excluded, because the principle of microscopic reversibility requires that the lowest-energy pathway for protonation of the carbanion be the reverse of the lowest-energy pathway for its formation. The exchange reactions result exclusively from general base catalysis by 3-quinuclidinone (see above), which shows that proton abstraction by the conjugate base of the solvent (i.e., DO^{-}) is a much higher energy pathway than proton abstraction by the buffer base.

Our data do not exclude deuterium exchange by a concerted mechanism in which deprotonation of ethyl thioacetate by 3-quinuclidinone and its reaction with a molecule of D_2O occur in a single reaction stage. However, there is no precedent for exchange of the α-protons of carbonyl compounds by such a mechanism.

We conclude that the rate constants k_{ex} for exchange of the first α-proton of each methyl group of ethyl thioacetate and acetone correspond directly to the rate constants for deprotonation of a single α-CH_3 group of the substrates by 3-quinuclidinone to give the *free* carbanions.

The observation that under our conditions there is very little hydrolysis of ethyl thioacetate competing with the 3-quinuclidinone-catalyzed carbon deprotonation reaction stands in sharp contrast to the estimate that hydrolysis is 5-fold faster than deprotonation when hydroxide ion acts as the base/nucleophile.[9] This confirms our prediction that the deprotonation of thiol esters should be increasingly favored relative to hydrolysis when the pH and the pK_a of the base are lowered.[10]

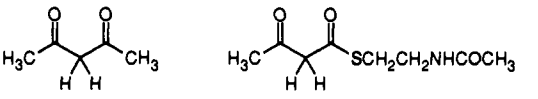
pK_a of Ethyl Thioacetate. The pK_a of a carbon acid can be calculated from the rate constants for its deprotonation by a base to give the free carbanion (k_B, Scheme IV) and for protonation of the free carbanion by the conjugate acid of the base to regenerate the substrate (k_{BH}, Scheme IV), according to eq 3. The

$$pK_a = pK_{BH} + \log (k_{BH}/k_B) \qquad (3)$$

3-quinuclidinone-catalyzed exchange reactions of ethyl thioacetate and acetone in D_2O proceed by the irreversible formation of the free carbanions (see above), so the rate constant k_{ex} for ethyl thioacetate gives $k_B = 2.2 \times 10^{-5}$ M^{-1} s^{-1} for deprotonation to give the free thiol ester enolate. However, the value of k_{ex} for acetone corresponds to the rate constant for deprotonation of only a single methyl group, so a 2-fold statistical correction of the value of k_{ex} gives $k_B = 5.2 \times 10^{-4}$ M^{-1} s^{-1} for the deprotonation of acetone to give the free enolate.[19]

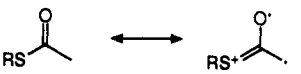
The value of $k_B = 5.2 \times 10^{-4}$ M^{-1} s^{-1} for deprotonation of acetone by 3-quinuclidinone and the values of $pK_a = 19$ for acetone[27] and $pK_{BH} = 7.5$ for 3-quinuclidinone[11b] in water can be substituted into eq 3 to give $k_{BH} = 1.7 \times 10^{8}$ M^{-1} s^{-1} for reaction of the free enolate of acetone with protonated 3-quinuclidinone. This analysis neglects the secondary solvent isotope effect on k_B arising from its determination here in D_2O rather than in H_2O, but a small solvent isotope effect of $k_B(H_2O)/k_B(D_2O) = 1.1$ has been determined for a similar system.[12a] The deprotonation of ethyl thioacetate by 3-quinuclidinone is 12-fold slower than the deprotonation of a single methyl group of acetone by the same amine base. Since the substituent effect on k_{BH} for the protonation of the two enolates is expected to be no larger than the substituent effect on the rates of their formation,[28] the rate constant for reaction of the free enolate of ethyl thioacetate with 3-quinuclidinone is estimated to be $k_{BH} = 1.7 \times 10^{8}$ to 2×10^{9} M^{-1} s^{-1}. The values of $k_B = 2.2 \times 10^{-5}$ M^{-1} s^{-1} and $pK_{BH} = 7.5$ for 3-quinuclidinone and the limits on k_{BH} can be substituted into eq 3 to give $pK_a = 20.4$–21.5 for ethyl thioacetate.

The 1.5–2.5-unit increase in pK_a that results when a methyl group of acetone is replaced by an ethylthio group is substantially larger than the corresponding substituent effect observed on the pK_as for **1** ($pK_a = 8.2$–8.9)[29] and **2** ($pK_a = 8.5$),[30] which have very similar acidities.[3a] This serves to emphasize the importance of



1 **2**

a comparison of the acidities of *simple* ketones and thiol esters, because the effects of electron-accepting substituents on the stability of an adjacent carbanion are smaller when it is already stabilized by other substituents, i.e., resonance is most important when the demand for it is greatest.[31] Our results suggest that the resonance overlap of the lone-pair electrons on the sulfur atom with the carbonyl group of the type **3**, which would tend to decrease the ease of formation of an adjacent carbanion, is more important than suggested previously.[3a,32]



3

Carbanion Lifetimes and Enzymatic Catalysis. The reaction of the enolate of acetone with protonated 3-quinuclidinone, $k_{BH} = 1.7 \times 10^{8}$ M^{-1} s^{-1}, is considerably slower than the diffusional encounter of the two species ($k_d = 5 \times 10^{9}$ M^{-1} s^{-1}), so this reaction is limited by the chemical barrier to the protonation of the enolate within the buffer acid–enolate ion pair $BH^{+}\cdot^{-}CH_2COMe$ (k_{-1}, Scheme III). This is consistent with the observation of substantial primary isotope effects on the deprotonation of acetone to give the enolate,[22,33] which require that the proton-transfer step (k_1, Scheme III) rather than diffusional separation of $BH^{+}\cdot^{-}CH_2COMe$ (k_{-d}, Scheme III) be rate-limiting for formation of the free carbanion. Similarly, the limits of $k_{BH} = 1.7 \times 10^{8}$ to 2×10^{9} M^{-1} s^{-1} for the reaction of the thiol ester enolate with protonated 3-quinuclidinone show that there is a chemical barrier to proton transfer within the ion pair $BH^{+}\cdot^{-}CH_2COSEt$, i.e., *the simple thiol ester enolate has a significant lifetime in the presence of the 3-quinuclidinone cation.*

How long is this lifetime? The relationship $k_{BH} = K_{as}k_{-1}$ ($K_{as} = k_d/k_{-d}$, Scheme III), with an association constant for formation

(24) The rate constants for proton exchange between protonated quinuclidines and water[25] are several orders of magnitude smaller than the estimated[26] rate constant for diffusional separation of a cation–anion ion pair, $k_{-d} = 10^{10}$ s^{-1}. Exchange of BH^{+} with a second molecule of BD^{+} is limited by the rate constant for diffusional encounter, $k_d = 5 \times 10^{9}$ M^{-1} s^{-1}, so at low concentrations of BD^{+}, diffusional separation of the ion pair will occur before there is significant exchange.

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(28) The values of $\beta \geq 0.5$ for general-base-catalyzed deprotonation of ketones[12] provide evidence for a "late" transition state, so substituent effects on the stability of the enolates will be expressed largely in the rate constants for their formation.

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of a cation–anion ion pair of $K_{as} \approx 0.3$ M^{-1},[26] can be used to estimate $k_{-1} = 6 \times 10^8$ to 7×10^9 s^{-1} for collapse of $BH^{+\cdot}$-CH_2COSEt by proton transfer to give $B{\cdot}CH_3COSEt$, so in the presence of a general acid of $pK_{BH} = 7.5$, the thiol ester enolate has an estimated lifetime ($1/k_{-1}$) from 10^{-9} to 10^{-10} s. If an enzyme provides stabilization of the thiol ester enolate relative to the thiol ester,[12a] then the lifetime of such carbanions in an enzyme active site may well be even longer than 10^{-9} s. These results provide evidence against the suggestion that enzyme-catalyzed Claisen condensation and related reactions proceed by concerted mechanisms[5,6,8] that are enforced, because in the presence of an acidic amino acid residue at the enzyme the intermediate enolate cannot exist for the time of even a single bond vibration (ca. 10^{-13} s).[6]

Enzyme catalysts often act to stabilize reactive carbanion intermediates,[12a,34] and the primary barrier which must be lowered in order for enzymatic catalysis of deprotonation at the α-carbonyl position of simple ketones and thiol esters to occur is the thermodynamic barrier to the formation of these unstable enolates.[12a] The 1.5–2.5-unit difference between the pK_a of a simple ketone and that of a simple thiol ester shows that protein catalysts must overcome a 2–3 kcal/mol larger thermodynamic barrier in order to deprotonate the latter carbon acid.

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Highly Cooperative Binding of Alkyl Glucopyranosides to the Resorcinol Cyclic Tetramer Due to Intracomplex Guest–Guest Hydrogen-Bonding: Solvophobicity/Solvophilicity Control by an Alkyl Group of the Geometry, Stoichiometry, Stereoselectivity, and Cooperativity

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Abstract: The resorcinol cyclic tetramer (**1**) binds methyl and *n*-octyl glucopyranosides via hydrogen-bonding in apolar organic media. The complexation behaviors of these two alkyl glucosides are markedly different from each other. Methyl glucoside (**2**), which is otherwise insoluble in $CHCl_3$ or CCl_4, is solubilized in that solvent upon formation of a 2:1 (host to guest) sugar-encapsulation complex with a remarkable β/α anomer selectivity. Octyl glucoside (**3**), on the other hand, is soluble in $CHCl_3$ and is bound to host **1** to give a 1:4 (host to guest) complex with only a low anomer selectivity. The four guest molecules are bound at the four unit hydrogen-bonding sites of the host with an exceptionally high cooperativity that arises from intracomplex guest–guest hydrogen-bonding involving the 5-CH_2OH and 2-OH groups of the adjacent glucoside molecules. The way to achieve a maximal hydrogen-bond network is discussed in terms of solvophobicity/solvophilicity control by an alkyl group of the geometry, stoichiometry, stereoselectivity, and cooperativity.

Introduction

Complexation of sugar derivatives in apolar organic media is a rapidly growing area of molecular recognition.[1] Unprotected monosaccharides can be solubilized in an apolar solvent upon formation of lipophilic complexes with a suitable host such as the resorcinol cyclic tetramer (**1**).[1a] Host **1** has a symmetric bowl-shaped aromatic cavity and four independent hydrogen-bonding sites (A–D) composed of a pair of OH groups. Lipophilic sugar derivatives such as sugar glycosides having a long alkyl chain can also be used as guests; they undergo complexation in homogeneous solutions.[1b] Both solubilization and homogeneous complexations are promoted by the hydrogen-bonding interaction. It is not well understood, however, how the polar host–guest interaction is affected by the solvophobicity/solvophilicity or the polar/apolar balance of the guest.

In the present work, we have studied the complexation of host **1** with methyl glucopyranoside (**2**) and *n*-octyl glucopyranoside (**3**) (Chart I). The methyl and octyl derivatives are insoluble or solvophobic and soluble or solvophilic, respectively, in an apolar solvent such as $CHCl_3$. We report here that the octyl derivative exhibits a remarkable cooperativity due to intracomplex guest–guest hydrogen-bonding. It is also shown that the difference in the intrinsic solubilities of **2** and **3** results in a dramatic alteration of their complexation behaviors.

Results

Solubilization of Methyl Glucopyranoside. Methyl β-D-glucopyranoside (**2**β), otherwise completely insoluble in CCl_4, was

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