h. The reaction mixture was washed with two 50-mL portions of ice-cold 1 N hydrochloric acid solution and two 50-mL portions of ice-cold 10% sodium hydroxide solution. The acidic, aqueous solution was basified with 10% sodium hydroxide solution, and starting hydroxylamine precipitated out. This material was collected on a Büchner funnel, washed with water, and sucked dry to give 0.41 g (20%) of recovered starting material. The etheral solution was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to a dark oil. The oil was recrystallized by addition of 5 times its volume of petroleum ether, and the mixture was cooled in a dry ice/2-propanol bath, filtered, washed with cold petroleum ether, cooled to -72 °C, and sucked dry to give 0.89 g (54% yield) of *O*-acetyl compound: mp 35-37 °C; NMR ($CDCl_3$) δ 8.77 (s, 9), 7.90 (s, 3), 2.68 (br s, 5); IR (neat) 2960, 1790, 800, 695 cm^{-1}.

***O*-Methyl-*N*-*tert*-butyl-*N*-phenylhydroxylamine.** A 0.0756-g (0.63 mmol) sample of potassium hydride (33% oil dispersion) was placed in a sintered-glass funnel and washed with two 50-mL portions of dry hexane, sucked dry under an atmosphere of dry nitrogen, and transferred with 50 mL of dry methyl sulfoxide into a flask, and 0.0981 g (0.55 mmol) of *N*-*tert*-butyl-*N*-phenylhydroxylamine was added. To this oil-free solution there was added 0.17 mL (1.02 mmol) of dimethyl sulfate. Upon addition of the dimethyl sulfate, the reaction mixture turned red. The mixture was allowed to stand at room temperature for 30 min, diluted with 500 mL of water, and extracted with three 100-mL portions of ether. The combined ethereal extracts were extracted twice with 50-mL portions of ice-cold 1 N hydrochloric acid solution and twice with equal volumes of water, dried over anhydrous sodium carbonate, filtered, and concentrated under reduced pressure to give a red oil. The red oil was purified by molecular distillation at 0.10 mm and 20 °C to give 0.0357 g (36%) of a red liquid containing *O*-methyl-*N*-*tert*-butyl-*N*-phenylhydroxylamine: NMR ($CDCl_3$) δ 8.88 (s, 9), 6.55 (s, 3), 2.73 (br s, 5); IR (neat) 2970, 1205, 770, 700 cm^{-1}. The NMR spectrum indicated it was 90% pure.

Registry No. 1, 618-40-6; 2, 36171-18-3; 3, 530-50-7; 4, 17223-85-7; *N*-methylphenylamine, 26915-12-8; *N*-*tert*-butylaniline, 937-33-7; diphenylamine, 122-39-4; carbazole, 86-74-8; *N*-nitroso-*N*-*tert*-butylaniline, 24642-84-0; nitrous oxide, 10024-97-2; methyl *tert*-butyl ether, 1634-04-4; *O*-acetyl-*N*-*tert*-butyl-*N*-phenylhydroxylamine, 76599-72-9; *N*-*tert*-butyl-*N*-phenylhydroxylamine, 1127-42-0; *O*-methyl-*N*-*tert*-butyl-*N*-phenylhydroxylamine, 76599-73-0.

Nuclear Magnetic Resonance, Paramagnetic Ion Induced Relaxation Method to Differentiate between 1,3-Diketo and 1,3-Keto-Enol Isomers[1]

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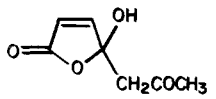
In aqueous solution manganous ion is shown to broaden the methyl ^1H resonance of the enol form of acetylacetone. Under the same conditions, where [Mn(II)] ≪ [keto–enol] and ≪ [1,3-diketone], the methyl of the diketo form is affected to a smaller degree. This is primarily due to the more favorable association constant of Mn(II) with the keto–enol than with the diketo form. This diagnostic is used to differentiate between resonances due to diketo and keto–enol forms of the substrate for maleylacetone cis–trans isomerase and is proposed for use in elucidating NMR spectra of other diketo/keto–enol mixtures.

Several years ago we reported the synthesis of maleylacetone ((*Z*)-4,6-dioxo-2-heptenoic acid),[2a] a substrate for the cis–trans isomerizing enzyme isolated from Vibrio 01 bacteria.[2b,e] The low-wavelength ultraviolet absorption maximum (195 nm) of the acid suggested that it exists in the cyclic form I, but at pH 7, strong absorption at 312 nm



I

(ε 9300) indicated that the 4-hydroxy-6-oxo-2,4-heptadienoate ion (IIa and/or IIb) was present. Absorption at 212 nm (ε ~4000) and 243 (~4000) suggested that the (*Z*)-4,6-dioxo-2-heptenoate ion (IIc) may also be present at neutral pH.[2a] Of the two enol forms, (2*Z*,4*E*)-4-hydroxy-6-oxo-2,4-heptadienoate ion (IIa) and (2*Z*,4*Z*)-4-hydroxy-6-oxo-2,4-heptadienoate ion (IIb), the presence of IIa might be thought to be more probable because of the intramolecular hydrogen bond. In solvent water, however, intermolecular hydrogen bonding may compensate for the loss of the intramolecular bond. Moreover, the presence of IIb was postulated, for it was seen to have a mechanistic role in the silver ion catalyzed cis–trans isomerization about the C-2,C-3 bond of II.[2c,d]

Although the presence of three species has been mentioned, it was known early that only two ^1H NMR methyl singlets of about equal intensities are exhibited by II in D_2O,[2a] even at 360 MHz. The two singlets are 0.08 ppm apart and are about equally intense. Two limiting interpretations are possible. The first is that one methyl singlet is due to the keto form IIc and one to a rapidly equilibrating mixture of IIa, IIb, and perhaps other enol isomers. This would mean that the molar extinction coefficient for the mixture of enol isomers at 312 nm would be unusually large (ε ~18 500). Moreover, interconversion of IIa and IIb would have to be more rapid than the conversion of either one to IIc. That is to say that a lower energy path than that shown in eq 1 is required to convert IIa to IIb.

The second possible interpretation is that IIc is present in very small quantity. Then one methyl singlet represents IIa and the other IIb, and the methyl resonance of IIc is undetectable or isochronous with one of the other resonances. Interconversion could occur according to eq 1 and

(1) Research carried out at Brookhaven National Laboratory under contract with the U.S. Department of Energy and supported by its Office of Basic Energy Sciences and by the National Institutes of Health (Grant GM 27636).

(2) (a) Fowler, J.; Seltzer, S. *J. Org. Chem.* **1970**, *35*, 3529. (b) Seltzer, S. *J. Biol. Chem.* **1973**, *248*, 215. (c) Johnson, R. A.; Seltzer, S. *J. Am. Chem. Soc.* **1972**, *94*, 4755. (d) Johnson, R. A.; Seltzer, S. *Ibid.* **1973**, *95*, 5700. (e) Seltzer, S.; Lin, M. *Ibid.* **1979**, *101*, 3091.

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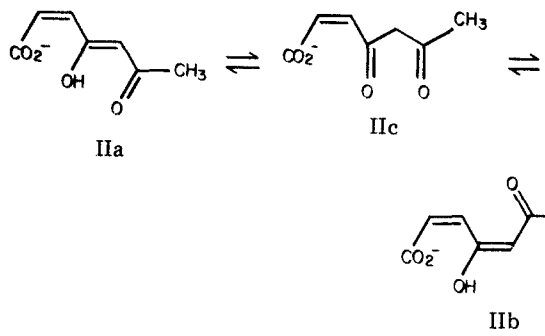


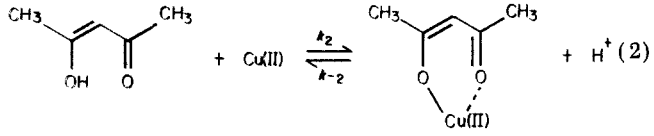
IIa IIc IIb (1)

would be slow on the NMR time scale.

^{13}C NMR data presented here, however, show that one of the methyl singlets is due to the keto form. ^{13}C NMR chemical shifts are in line with those observed for the enol and keto forms of acetylacetone.

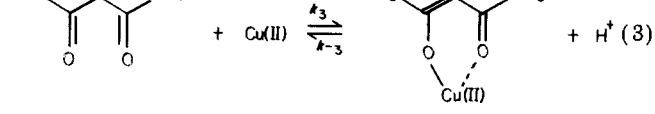
Nevertheless, two relatively stable isomers of the substrate are present in neutral aqueous solution; it is likely that the enzyme acts upon only one. If this is true, it would be of interest to determine the complimentary structure of the enzyme by determining the structure of the isomer that it accepts. NMR studies to help answer this are underway, but it is first necessary to assign each methyl singlet to the correct isomer of the substrate. We describe here a hyperfine relaxation method which we have used to assign the methyl singlets of II and which has obvious use in elucidating NMR spectra of other keto/enol mixtures.

In aqueous solution, the reaction of Cu(II) with acetylacetone to form the (acetylacetonato)copper(II) ion exhibits a fast and slow rate. The fast rate has been shown to be due to the association of the enol with Cu(II) (eq 2)



(2)

and the slow rate to the reaction of the keto form and Cu(II) (eq 3).[3] From stopped-flow measurements carried



(3)

out in aqueous acid at 25 °C the second-order formation rate constant for the keto form (eq 3) is about 10 M^{-1} s^{-1} while for the enol form (eq 2) it is 2×10^4 M^{-1} s^{-1}.[3a] The large difference in rate is presumably due to the slower rate of deprotonation from carbon compared to loss from oxygen. The fast rate of association exhibited for reaction of Cu(II) with the enol form has been suggested to be also characteristic for two other divalent metal ions, viz., Mn(II) and Co(II), in forming six-membered chelates.[3a] Manganous ion, fortunately, has a relatively long electron-spin relaxation time.[4] Consequently, molecules that bind manganous ion could be expected to have their nuclear spins, in the neighborhood of the binding site, relaxed. If, as for Cu(II), Mn(II) were to react much faster with the enol than with the keto form, we would anticipate that

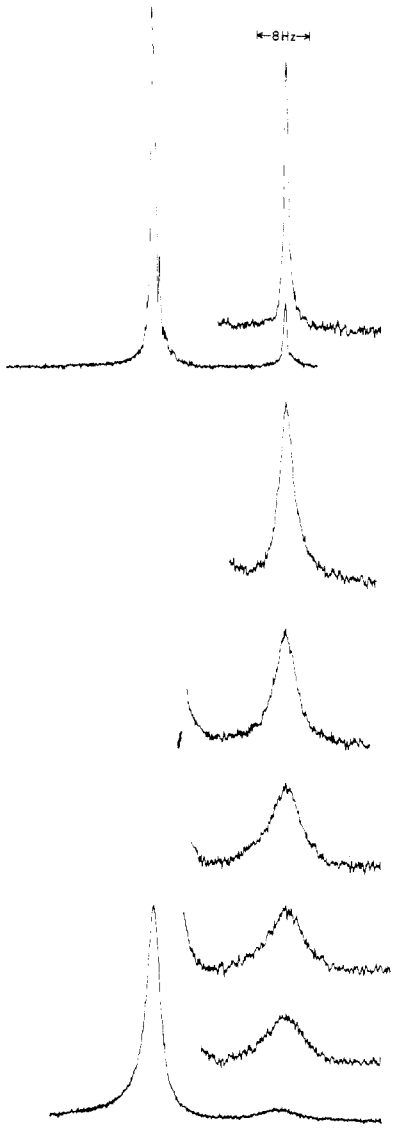


Figure 1. ^1H NMR spectra of acetylacetone (0.2 M) in D_2O at pD 4.88. From top to bottom the Mn(II) concentrations are 0, 8.43×10^{-4}, 1.65×10^{-3}, 2.43×10^{-3}, 3.19×10^{-3}, and 4.26×10^{-3} M.

Mn(II) would have a more profound effect on the methyl resonance of the enol form than the keto form, and this difference could be used to assign resonances to the enol and keto forms of 1,3-diketo systems.

In this paper we show that the relaxing effect of Mn(II) on ^1H nuclear spins of 1,3-keto–enol molecules is indeed considerably larger than the corresponding effect on 1,3-diketo systems. The different behavior for the two systems, however, on the NMR time scale, appears to be due to differences in Mn(II) association constants for 1,3-diketo and 1,3-keto–enol systems and not due to differences in rates of formation of these complexes.

Experimental Section

^1H NMR studies were carried out at 100 MHz with a JEOL MH-100 and with a Varian FT-80 at 80 MHz. The probe temperature was maintained with a JEOL temperature controller and was calibrated by measuring the chemical shift difference between hydroxyl and methyl protons in methanol and between hydroxyl and methylene protons in 1,3-propanediol. ^{13}C NMR studies were carried out with a Bruker spectrometer at 90.52 MHz and with a Varian CFT-20 at 20 MHz. Acetylacetone (Fisher Scientific) was reagent grade and used as supplied. 3,3-Dimethyl-2,4-pentanedione (ICN-K and K Laboratories) was used as supplied. Maleylacetone was synthesized as described previously.[2] Man-

(3) (a) Pearson, R. G.; Anderson, O. P. *Inorg. Chem.* **1970**, *9*, 39. (b) Taft, R. W., Jr.; Cook, E. H. *J. Am. Chem. Soc.* **1959**, *81*, 46. (c) Celiano, A. V.; Cefola, M.; Gentile, P. S. *J. Phys. Chem.* **1961**, *65*, 2194. (d) Fay, D. P.; Nichols, A. R., Jr.; Sutin, N. *Inorg. Chem.* **1971**, *10*, 2096.

(4) James, T. L. "Nuclear Magnetic Resonance in Biochemistry"; Academic Press: New York, 1975, pp 174–181.

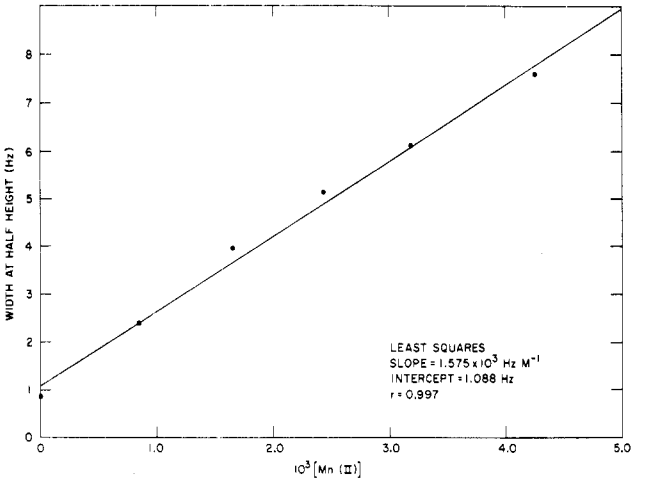


Figure 2. Widths at half-height of the upfield methyl of acetylacetone vs. the total Mn(II) concentration present.

Table I. Slopes and Intercepts from Plots of Upfield Methyl Peak Widths at Half-Height of Acetylacetone vs. Mn(II) Concentration at Different Temperatures

temp, °C	10^{-3}(slope), Hz M^{-1}	int, Hz
3.8	1.38	0.87
19.8	1.58	1.09
39.5	1.44	2.0

ganous nitrate (Matheson Coleman and Bell; 50% solution) served as the source of manganese. Dilute manganous nitrate solutions (0.86 to 430 mM) in D_2O were prepared. D_2O was distilled before use. Deconvolution of spectra was carried out with a Du Pont curve resolver. The ^1H NMR peak shape for acetone served as the prototype peak shape and was introduced into the curve resolver's function-generator channels.

Results

The two ^1H NMR methyl singlets of the maleylacetone conjugate base, which are observed near neutral pH, collapse to a single peak at high and low pH. For this reason all NMR studies were carried out as close to pH neutrality as could be achieved without precipitating manganese hydroxide. Studies were first carried out with acetylacetone to demonstrate that manganous ion would collapse the methyl of the enol form without substantially affecting the methyl of the keto form. Figure 1 shows the shape of the methyl resonances of acetylacetone in D_2O after addition of small amounts of Mn(II) to a constant concentration of acetylacetone (0.2 M) at 20 °C. In this study, which is typical, the highest concentration of Mn(II) used was 4.2 mM, ensuring that the concentration of acetylacetone was greater than the paramagnetic ion concentration. In water the enol form amounts to 15% of the total acetylacetone.[5] At the top of Figure 1 are the methyl peaks for the keto and enol forms. Below are represented the methyl singlets of the enol form arranged in the order of increasing Mn(II) concentration. At the bottom are the methyl singlets for keto and enol forms with the highest Mn(II) concentration present. It can be seen at a glance that the enol methyl singlet is affected much more strongly by added manganese than the methyl singlet of the keto form. The full width at half-height of each methyl singlet of the enol form, shown in Figure 1, was measured, and each width is shown plotted in Figure 2 against the corresponding Mn(II) concentration. Similar experiments were carried out at 3.8 and 39.5 °C, and the resulting slopes and intercepts are shown in Table I. When the Mn(II) concentration was increased further, the methyl singlet of the keto form continued to broaden. The results of least-squares fitting are shown in Table II. In addition, the experiments with acetylacetone and manganous ion were repeated at other pD's, and these are also listed in Table II. In all cases the statistics were quite good as shown by the correlation coefficient. Over the range of manganous ion concentration used, the keto methyl singlet was seen to move ~0.02 ppm to lower field relative to internal DSS as a reference.

Similar experiments were carried out with maleylacetone and typical results are shown in Figure 3. As the concentration of Mn(II) is increased, both peaks appear to broaden. The two singlets overlap, and for the observance of the effect of Mn(II) on the individual peaks a Du Pont 310 curve resolver was used to deconvolute the methyl part of the spectrum. The peak shape of an acetone solution under similar conditions was recorded, and this served as

(5) Schwarzenbach, G.; Felder, E. *Helv. Chim. Acta* **1944**, *27*, 1044.

Table II. Linear Dependence of the ^1H NMR Methyl Peak Width at Half-Height vs. the Manganous Ion Concentration

entry	ketone (form)	concn[b]	pD	least-squares slopes, Hz M^{-1}[a]
	Unbuffered			
1	acetylacetone (E)[b]	0.070	6.4	9.6×10^3
2	acetylacetone (K)[b]	0.37	5.8	5.4×10^2
3	acetylacetone (E)[b]	0.036	4.9	1.6×10^3
4	acetylacetone (K)[b]	0.16	3.3	4.1×10^2
5	maleylacetone (E)[b]	0.032	6.4	4.9×10^4
6	maleylacetone (K)[b]	0.060	4.6	7.2×10^2
7	3,3-dimethyl-2,4-pentanedione	0.36	3.6	5.2×10^2[c]
8	acetone	1.0	5.6	4.8×10^2
	Buffered Solutions[d]			
9	acetylacetone (K)[b]	0.17	7.7	8.43×10^2
10	acetylacetone (E)[b]	0.17	7.7	1.18×10^4
11	acetylacetone (K)[b]	0.18	5.4	6.02×10^2
12	acetylacetone (E)[b]	0.17	5.4	3.43×10^3
13	acetylacetone (E)[b]	0.31	5.5	3.44×10^3
14	acetylacetone (K)[b]	0.77	5.4	6.79×10^2
15	3,3-dimethyl-2,4-pentanedione	0.18	5.4	6.1×10^2[e]
16	acetone	0.18	5.4	6.5×10^2

[a] Correlation coefficients (r) of the least-squares slopes and intercepts with the observed data for the systems in the table range from 0.984 to 0.999. The millimolar concentrations of Mn(II) used for each slope determination are as follows: (1) 0.0, 0.132, 0.262, 0.390, 0.515; (2) 0.0, 3.98, 7.89, 11.7, 15.5; (3) 0.0, 0.843, 1.65, 2.43, 3.19, 4.26; (4) 0.0, 4.26, 8.43, 12.5, 16.5, 30.0; (5) 0.0, 0.0200, 0.0391, 0.0573, 0.0832, 0.123; (6) 0.0, 1.76, 3.47, 5.12, 6.72, 8.27; (7) 0.0, 2.50, 4.89, 7.17, 9.35; (8) 0.0, 2.50, 4.89, 7.17, 9.35; (9) 0.0, 0.827, 1.62, 2.39, 3.13, 3.84, 4.53; (10) 0.0, 0.0923, 0.182, 0.268, 0.351, 0.432; (11) 0.0, 0.843, 1.65, 2.43, 3.19, 4.95; (12) 0.0, 0.384, 0.754, 1.11, 1.46, 1.79; (13) 0.0, 0.353, 0.694, 1.02, 1.34, 1.65; (14) 0.0, 0.768, 1.51, 2.22, 2.92, 3.91, 4.86; (15) 0.0, 0.824, 1.62, 2.38, 3.47, 4.51, 5.49; (16) 0.0, 0.558, 1.10, 1.63, 2.15, 2.90, 3.63, 4.78. [b] K = 1,3-diketone form; E = 1,3-keto-enol form. The concentration given is for the concentration of that tautomer. [c] The upfield methyl (δ 1.15) exhibits a corresponding broadening with a slope of 5.10×10^2 Hz M^{-1}. [c] Buffered solutions in the range of pD 5.5 were prepared from 0.20 M acetic acid-d_4 and partially neutralized with NaOD in D_2O. Buffered solutions in the pD 7.7 range were prepared from partially acidified (DCl) 0.2 M imidazole in D_2O. [e] These data are for the keto methyl peak at δ 2.11 ($CDCl_3$, internal Me_4Si). The upfield methyl (δ 1.15) exhibits a corresponding broadening with a slope of 6.0×10^2 Hz M^{-1}.

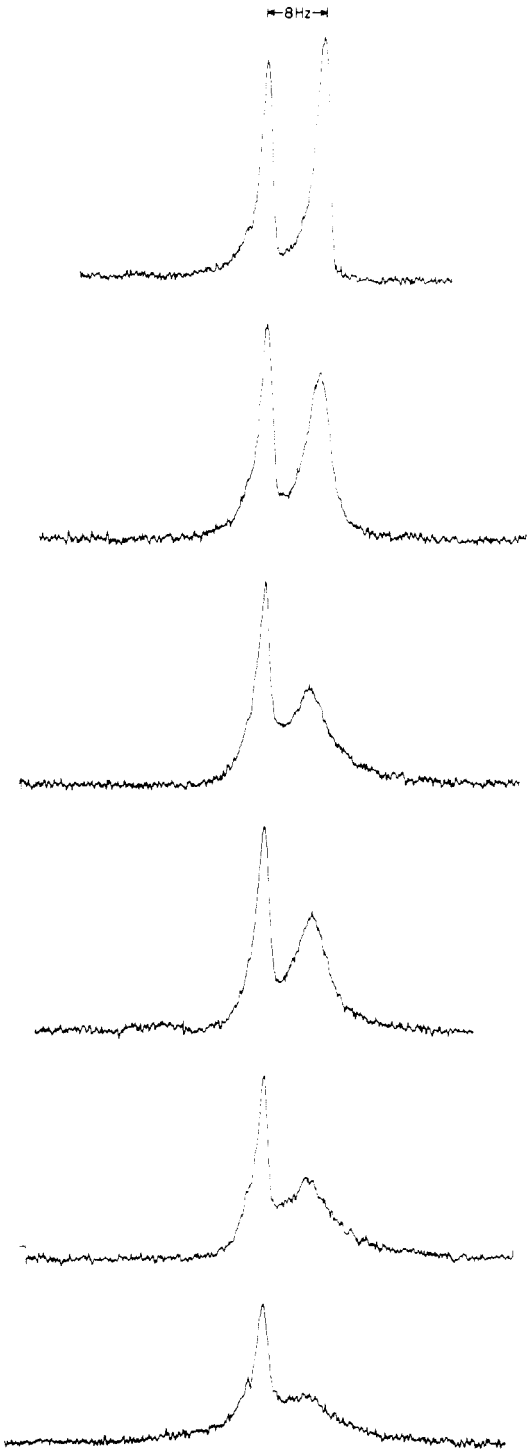


Figure 3. ^1H NMR spectra of the methyl region of maleylacetone (0.061 M) in D_2O at pD 6.4. From top to bottom the Mn(II) concentrations are 0, 2×10^{-5}, 3.91×10^{-5}, 5.73×10^{-5}, 8.32×10^{-5}, and 1.23×10^{-4} M.

the prototype for the function generators of the curve resolver. The widths at half-height of the upfield methyl resonance, extracted from the deconvoluted spectra, are shown in Figure 4 plotted against the corresponding Mn(II) concentration. The deconvoluted downfield methyl has a relatively constant width at half-height over the range of manganous ion used in the study of its effect on the upfield methyl (Figure 4). The mean width of the downfield methyl peak over the range of 0.0 to 1.2×10^{-4} M manganous ion in all of the spectra is 1.8 Hz with an average deviation of 0.1 Hz. A further increase in the manganous concentration results in the broadening of the downfield methyl. The dependence of its broadening on

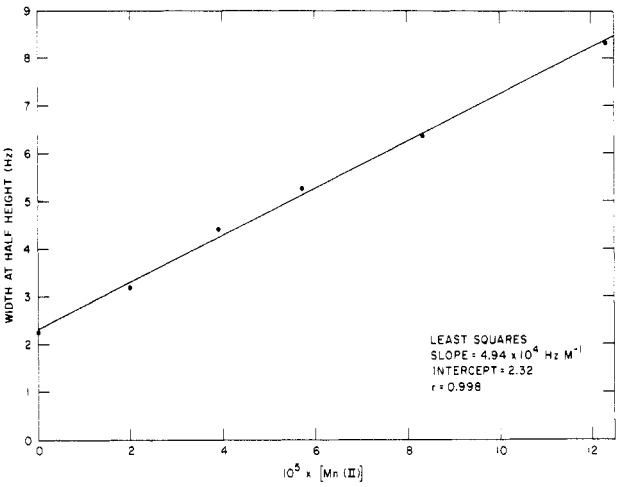


Figure 4. Widths at half-height of the upfield methyl of maleylacetone vs. the total Mn(II) concentration present (pD 6.4).

Table III. ^{13}C Chemical Shifts of Maleylacetone[a] and Aceylacetone in H_2O[b]

	shift, δ				
		acetyl-acetone[c]		acetyl-acetone[d]	
assignment	maleyl-acetone	enol	keto	enol	keto
CH_3	30.97, 33.55	24.69	33.05	24.3	30.2
$O{=}CCH_2C{=}O$	59.14		59.76		58.2
$COH{=}CHC{=}O$	107.16	100.29		100.3	
vinyl carbons	129.44, 131.50, 137.83, 142.18				
CO_2^-	174.81, 177.49				
=COH and/or C=O of enol form	198.37	191.03		191.0	
carbonyl carbons of keto form	204.38, 211.08		180.81		201.9

[a] At 4 °C; the solvent contained 10% D_2O; measurements were taken at pH 6.24. [b] External D_2O served as the lock solvent. [c] In H_2O at 20 MHz. [d] In $CDCl_3$; see ref 10.

the concentration is shown in Table II.

Similar studies were carried out with acetone and 3,3-dimethyl-2,4-pentanedione. As in the studies above, an excellent linear dependence of the widths at half-height on the manganous ion concentration was found. The least-squares slopes are shown in Table II together with those for the acetylacetone and maleylacetone systems.

In the studies just described aqueous solutions of ketones were unbuffered to minimize any complication that might arise due to association between Mn(II) and the base form of the buffer. Under these conditions, however, it is difficult to produce the same pD's from one system to the next. Additional studies were carried out with 0.20 M buffered solutions of acetate and imidazole at pD 5.4 and 7.7, respectively. In these separate studies the concentrations of the keto and enol forms of acetylacetone, 3,3-dimethyl-2,4-pentanedione, and acetone are the same (0.17–0.18 M) at pD 5.4. The slopes are given in Table II. Table II also records the effect of Mn(II) concentration on the NMR line widths of enol and keto forms of acetylacetone at the same concentration (0.17 M) but at pD 7.7 as well as at higher concentrations of the enol and keto forms at pD 5.4.

^{13}C NMR spectra were recorded for acetylacetone and maleylacetone (pH 6.24) in H_2O. The measured chemical shifts together with those published for acetylacetone are shown in Table III. During the time required to accumulate a sufficient number of transients for a ^{13}C spectrum, even at 90.52 MHz, cis–trans isomerization about the C-2,C-3 double bond proceeds to a significant extent. Because of this lability, similar ^{13}C NMR studies of the interaction of Mn(II) with maleylacetone are impractical because of the limited amount of substrate available. Therefore, parallel ^{13}C NMR studies with Mn(II) as described above for ^1H NMR were not attempted.

Discussion

As anticipated, the effect of added manganous ion in relaxing nuclear spins of the enol form of acetylacetone is more pronounced than its relaxing effect on the keto form (see Figures 1 and 2 and entries 1–4 and 9–14 of Table II). It can be seen that the magnitude of the broadening effect (i.e., the slopes of Table II) is relatively independent of the concentration of ligand as long as the ligand concentration is much greater than that of added Mn(II). A fourfold change in the concentration of the diketo form of acetylacetone at pD 5.4 results in less than a 13% change in the magnitude of the broadening effect due to Mn(II) (Table II, entries 11 and 14) while a twofold change in the concentration of the enol form results in no change in the broadening effect slope (entries 12 and 13). Furthermore, the broadening effect on the enol form increases with increasing pD (Table II, entry 1 vs. 3 and 10 vs. 12) by a substantially larger factor than the corresponding change of the keto methyl (Table II, entry 2 vs. 4 and 9 vs. 11). Some studies were carried out in unbuffered systems; others were made with acetate and imidazole buffers. Acetate and imidazole exhibit relatively low association constants toward manganous ions.[6] Comparison of the broadening effect of Mn(II) on keto and enol forms of acetylacetone in the presence and absence of buffer suggests little or no perturbation upon addition of these buffers.

At equal keto and enol isomer concentrations and at pD 7.7, the enol form is 14 times more sensitive to nuclear spin relaxation by manganous ion than is the keto form. This does not appear to be an effect of a faster rate of association of metal and ligand but rather a more favorable chelate formation constant for the enol as compared to the keto form (vide infra). At pD 4.9 where one set of measurements of the enol methyl half-width vs. Mn(II) concentration was made, the concentration of enolate anion in equilibrium with the enol form of acetylacetone is very low, and, consequently, the major amount (79.8%) of the total Mn(II) added exists as the unchelated aquo complex (see Appendix I). A small amount (19.8%) exists as the acetylacetone–Mn(II) complex, and a negligible amount (0.4%) exists as the bis(acetylacetone)–Mn(II) complex. In the type of study shown in Figures 1 and 2, the concentration of ligand is about 50 times greater than the highest concentration of manganous ion used. Even if account is taken of the lower concentration of the enol form present, the concentration of acetylacetone–Mn(II) complex is only about 2% of the total free enol form present. Thus it is apparent that collapse of the methyl resonance by added Mn(II) must be the result of rapid chemical exchange. That is, each paramagnetic ion comes in contact with several acetylacetone molecules during the NMR time frame.

Swift and Connick[7] have derived an expression (eq 4)

$$\frac{1}{T_2} = \frac{1}{T_{2_A}} + \frac{P_B}{\tau_B}\left[\frac{\frac{1}{T_{2_B}^2} + \frac{1}{T_{2_B}\tau_B} + \Delta\omega_B^2}{\left(\frac{1}{T_{2_B}} + \frac{1}{\tau_B}\right)^2 + \Delta\omega_B^2}\right] \quad (4)$$

which relates the observed spin–spin relaxation time (T_2) for a nucleus of a ligand in the presence of chemical exchange between the free ligand and metal coordinated ligand to the chemical shift difference between the two sites in radians/second ($\Delta\omega_B$), the lifetime of the metal–ligand complex (τ_B), the spin–spin relaxation time in the metal ligand complex state (T_{2_B}), and the spin–spin relaxation time (T_{2A}) in the free ligand form. P_{B_1} is the mole fraction of the enol ligand coordinated to the metal and is essentially given by $m/(m + a + (H^+)/K_{ass_1})$ where m is the total manganous ion concentration in the system, a is the total acetylacetone enol form concentration, and K_{ass_1} is the first-stage association equilibrium constant (see Appendix II).

Simplification of eq 4 requires some knowledge of the relative magnitude of the $T_{2_B}^{-1}$, τ_B^{-1}, and $\Delta\omega_B$ terms. In general, in relaxation studies with Mn(II) little or no change in the chemical shift of the ligand has been observed when it is bound to Mn(II).[4] In this study we have observed only a very small downfield shift at the highest Mn(II) concentration (0.03 M) studied. So it would appear that the $\Delta\omega_B$ term in this system is negligible. Under these conditions eq 4 reduces to eq 5. The relative importance

$$\frac{1}{T_2} = \frac{1}{T_{2_A}} + \frac{P_B}{T_{2_B} + \tau_B} \quad (5)$$

of $T_{2_B}^{-1}$ and τ_B^{-1} is often determined from the temperature dependence of the variation of line width with concentration of the paramagnetic species.[3a] The width at half-height ($w_{1/2}$), measured in hertz, is a measure of the transverse relaxation time (T_2); $\pi w_{1/2} = T_2^{-1}$. τ_B is the lifetime of the chelate complex, and thus τ_B^{-1} is the rate constant for dissociation of the complex. Consequently, τ_B^{-1} is expected to show a normal temperature dependence, that is, a minimum Arrhenius activation energy of ~3 kcal/mol. Thus if τ_B^{-1} were the dominant term in eq 5, the slope derived from an experiment as illustrated in Figure 2 (i.e., T_2^{-1} vs. P_B) would be expected to increase with increasing temperature. The temperature dependence on $T_{2_B}^{-1}$, however, is generally small and often shows an apparent negative activation energy. Therefore, if $T_{2_B}^{-1}$ were dominant, the variation of half-width with manganous ion concentration would show little or no temperature dependence.

The variation of half-widths of the enol methyl peak with manganous ion concentration was studied at three temperatures over a 35 °C temperature range. The results as shown in Table I indicate essentially complete temperature independence over the temperature span studied. We conclude that dissociation of the complex cannot be rate controlling. Thus $\Delta\omega_B^2 \ll T_{2_B}^{-2}$ or τ_B^{-2}, and $\tau_B^{-2} \gg T_{2_B}^{-2}$. Under these conditions, eq 5 reduces to eq 6.[4]

$$\frac{1}{T_2} = \frac{1}{T_{2_A}} + \frac{P_B}{T_{2_B}} \quad (6)$$

(6) Martell, A. E.; Smith, R. M. "Critical Stability Constants"; Plenum Press: New York, 1977; p 5. Sillen, L. G.; Martell, A. E. *Spec. Publ.—Chem. Soc.* **1964**, *No. 17*, 388. Sillen, L. G.; Martell, A. E. *ibid.*, **1971**, *No. 25*, 281.

(7) Swift, T. J.; Connick, R. E. *J. Chem. Phys.* **1962**, *37*, 307.

Widths at half-height are shown plotted against m in Figure 2 for the methyl of the enol form. Similar least-squares solutions of the linear plots for the methyl of the keto form of acetylacetone are shown in Table II. If $a \gg m$,[8] eq 6 can be written for the enol form as in eq 7. The slope of such a plot is therefore equal to $(\pi[a + (H^+)/K_{ass_1}]T_{2_B})^{-1}$.

$$w_{1/2} = (\pi T_{2_A})^{-1} + m/(\pi[a + (H^+)/K_{ass_1}]T_{2_B}) \quad (7)$$

Previous reports of the kinetics of the reaction of acetylacetone with Cu(II)[3a] and with Fe(II)[3d] by stopped-flow methods discuss the formation of a common mono bidentate ligand complex arising from either the enol or keto form (eq 2 and 3). As already mentioned, the rate constant for formation from the enol and Cu(II) (2×10^4 M^{-1} s^{-1}) is much greater than for that formation from the keto form (10 M^{-1} s^{-1}). The latter reaction is considerably slower because the rate-controlling step requires transfer of a proton from carbon to water. If a common intermediate were in rapid reversible equilibrium with enol and keto forms, it would seem difficult to rationalize the presence of separate enol and keto ^1H NMR methyl peaks after the addition of manganous ion. If association constants of Mn(II) with the two ligands were almost equal and rates of association–dissociation were rapid, then the two peaks should be expected to merge as they broaden. If however, association with the enol is considerably more favorable and faster than with the keto form, then dissociation of the common intermediate has to be mainly to the enol form to maintain the equilibrium. If the association constants are substantially separated, it is possible then for reversible reaction to take place between Mn(II) and the enol form at low concentrations of Mn(II), broadening the enol methyl peak, without substantially affecting the keto form. As the manganous ion concentration is increased, complete collapse of the enol methyl could take place without causing the peaks to merge if association with the keto form at that concentration of manganous ion were negligible. A continued increase in the manganous ion concentration would lead finally to the broadening of the keto methyl peak with simultaneous shifting of its position to the weighted average position, and this may be difficult to sort out.

Another reasonable explanation has been suggested.[9] This is that on the NMR time scale, a complex between Mn^{II} and the diketo tautomer stays together for a sufficient length of time for some nuclear relaxation to take place but for an insufficient time for loss of a proton from carbon. Consequently, the complex formed from the enol form will be different from that from the keto form. The enolate complex returns mainly to enol form and the Mn–keto complex returns to diketo form.

For investigation of this point the interaction of Mn^{II} with a nonenolizeable analogue of acetylacetone, 3,3-dimethyl-2,4-pentanedione, was studied. Indeed, the methyl resonances of a dilute aqueous solution of 3,3-dimethyl-2,4-pentanedione in D_2O are similarly broadened by added manganous ion. As shown in Table II (entries 11 and 15), the slope of the linear dependence of half-width vs. manganous ion concentration is essentially the same as it is for acetylacetone at the same concentration of diketone and the same pD. These results strongly suggest that a transient complex of manganous ion and the diketo form of acetylacetone lives long enough for paramagnetically induced nuclear relaxation to take place. It appears that loss of a proton from the central carbon to attain the more stable and probably longer lived manganese–enolate complex is not necessary to attain relaxation. Furthermore, were it necessary to form the manganese–enolate complex from the diketo form, one might expect that a substantial change in the sensitivity of the keto methyl peak width to added manganese would be observed with a change in pD. That there is only a 20–40% change over ~2.4 pD units (entries 2 vs. 4 and 9 vs. 11) again suggests that proton loss from C-3 does not take place within the NMR time frame. Thus for the diketo system the parallel expression of eq 8 described the dependence of peak width

$$w_{1/2} = (\pi T_{2_A})^{-1} + m/(\pi[k + 1/K_{ass_2}]T_{2_B}) \quad (8)$$

at half-height as a function of total manganous ion concentration, m, total diketo form concentration, k, and the association constant, K_{ass_2}, between Mn(II) and the diketone (see Appendix II).

Moreover, it would appear that this transient complex need not be one where both oxygens of acetylacetone have to associate simultaneously with manganous ion. Acetone was also subjected to varying concentrations of manganous ion in D_2O. The methyl peaks also exhibit broadening over the same range of concentration of paramagnetic ion. As seen in Table II, the sensitivity exhibited by acetone is about the same as that for 3,3-dimethyl-2,4-pentanedione.

Why then is the slope for the relaxation of the methyls of the enol form greater than for the keto form? As shown by the temperature measurements, these effects are the effects of rapid equilibrium, and, consequently, the greater effect for the enol form must mean a greater association constant of Mn(II) with enol species. Therefore, a lower concentration of paramagnetic species is required to collapse the enol methyl peaks.

It is interesting that the broadening effect for the enol methyl is sensitive to the pD. We interpret this to mean that there is an increase in the association constant between the enol and Mn(II) at the higher pD, and for this to be realized in these experiments, the enol proton in the transitory complex has to be released to solvent within the NMR time frame.

After testing the method with known systems, we next turned to maleylacetone (II). As shown in Figure 3, two methyl singlets of almost equal intensity are observed. Because of the uncertainty of whether one of the predominant forms is the keto form (IIc), the ^{13}C NMR spectrum was recorded at 90.52 MHz in H_2O. The chemical shifts are shown in Table III together with those for acetylacetone for comparison. The most striking differences between the acetylacetone enol and keto spectra are the peaks at about 60 ppm for the diketo form and one peak at about 100 ppm for the enol form. The former is clearly due to the methylene carbon flanked by the two carbonyls in the keto form while the latter is surely C-3 of the enol form.[10] The positions of the other methyl and carbonyl carbon resonances of enol and keto forms are too close to each other to assign them definitely to one or the other tautomer on the basis of chemical shift alone. In solvents where the tautomeric ratio deviates markedly from unity as in water or chloroform, however, the intensities of the resonances permit unambiguous assignment. As shown in Table III, an aqueous solution of maleylacetone also exhibits single resonances in the 60- and 100-ppm regions which are strong and of about equal intensities.

(8) Upon increasing the concentration of Mn(II) ion, m may approach the value of a. Under this condition eq 7 may not hold, and a plot of width vs. manganous ion concentration may show systematic curvature.

(9) We are indebted to a referee for suggesting this possibility.

(10) Johnson, L. F.; Jankowski, W. C. "Carbon-13 NMR Spectra"; Wiley-Interscience: New York, 1972; No. 115.

By analogy we assign the 59.1-ppm peak to the methylene group of IIc and the 107.2-ppm peak to C-6 of the enol form (IIa and/or IIb). Other resonances for the carbons of methyls, vinyl, carbonyls, and carboxyl groups are in positions commonly found, and their assignments are as shown in Table III. The conclusion then is that one methyl ^1H NMR singlet of an aqueous solution of maleylacetone is due to the keto form (IIc) and the other to the enol form (IIa and/or IIb).

The effect of added Mn(II) on the ^1H NMR methyl signals of maleylacetone was then studied. In Figure 3 are shown the methyl singlets for a constant concentration of maleylacetone at pD 6.4 but in the presence of different concentrations of Mn(II). As seen, the upfield methyl signal appears to broaden more rapidly with added Mn(II) than the downfield methyl signal. Over the concentration range used for Mn(II), peak positions remain fixed, and thus this appears to be a case of rapid exchange as in the case of acetylacetone. While the downfield methyl also appears to broaden, the contribution of the very close broad upfield peak has to be reckoned with. This was accomplished with a Du Pont curve resolver. Deconvolution with the curve resolver reveals that the upfield singlet broadens progressively while the width of the downfield methyl remains constant and relatively narrow (1.8 ± 0.1 Hz) over the range of manganese concentrations used to study the broadening of the upfield methyl peak. The width at half-height for the deconvoluted upfield methyl plotted against the manganous ion concentration yields a very good straight line with a slope of 4.9×10^4 Hz M^{-1} (Figure 4). Unfortunately, concomitant irreversible cis–trans isomerization of II, especially rapid at higher temperatures, precludes us from carrying out NMR variable-temperature studies, but this system is sufficiently similar to the manganese–acetylacetone system for one to conclude that the broadening due to manganous ion is described by eq 7.

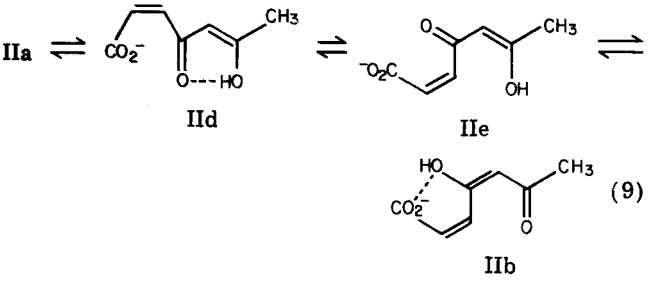
At much higher manganous ion concentrations the downfield methyl peak of maleylacetone broadens. A very good linear relationship of the width at half-height vs. the manganous ion concentration is found and has a slope of 7.2×10^2 Hz M^{-1} (Table II). This value when compared to others listed in Table II suggests that the downfield methyl is due to the keto form of maleylacetone. The sensitivity of broadening due to manganese in this system is somewhat higher. It is about 50% greater than for acetylacetone and about 40% greater than for acetone or 3,3-dimethyl-2,4-pentanedione, but this can be rationalized by suggesting that the nearby carboxylate group enhances association of the molecule with manganese and this leads to the somewhat greater slope.

The upfield methyl peak of maleylacetone more clearly corresponds to the enol form. The slope (Table II) seen here is about 5-fold greater than that observed for the enol form of acetylacetone. Here too, the neighboring carboxylate group could be acting to increase the association constant with manganese.

That a marked difference is observed between the effect of manganous ion on the methyl peaks of keto and enol forms of both acetylacetone and maleylacetone suggests that this method can be used as a useful diagnostic test to assign NMR resonances to enol and diketo tautomers.

A question remains as to the interconversion of IIa and IIb by a route which does not involve IIc.

As pointed out above the presence of the enol form, IIb, having the 2Z,4Z structure has been implicated in the mechanism of the silver ion catalyzed isomerization of II. We would anticipate, however, that the enol isomer IIa (2Z,4E) would be of lower energy than IIb because of the intramolecular hydrogen bond between oxygens at C-4 and C-6. Hence if only one methyl resonance is seen for the two enol isomers and one for the keto tautomer, there must be a path for rapid interconversion of IIa and IIb which does not involve IIc. This can be accomplished as shown in eq 9. Conversion of IIa to IIb requires movement of



the proton over a short distance along the hydrogen bond of IIa. Internal rotation about a C–C single bond converts IId to IIe, and removal of a proton from oxygen at C-6 of IIe by water and reprotonation of oxygen at C-4 by H_3O^+ would convert IIe to IIb. In the overall conversion, protons are removed or added to oxygen atoms, and a rotation about a single bond is required. These steps require considerably less energy than removing a proton from carbon as would be required by a pathway shown in eq 1. A similar rapid conversion of enol forms of acetylacetone may also be operating.

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Appendix I

The first- and second-stage Mn(II)–acetylacetone formation constants are given as eq A1 and A2, where E^- is

$$Mn^{2+} + E^- \rightleftharpoons MnE^+ \quad (K_{f_1} = 1.6 \times 10^4\ M^{-1}) \quad \text{(A1)}$$

$$MnE^+ + E^- \rightleftharpoons MnE_2 \quad (K_{f_2} = 1.23 \times 10^3\ M^{-1}) \quad \text{(A2)}$$

the enolate anion.[11] The overall acid dissociation constant of acetylacetone (K_a) is 1.02×10^{-9} M.[11] The concentrations of the various species are given by eq A3-A5, where

$$\frac{[Mn^{2+}]}{m} = \frac{(K_{f_1}K_{f_2})^{-1}}{d} \quad \text{(A3)}$$

$$\frac{[MnE^+]}{m} = \frac{aK_a(K_{f_2})^{-1}/((H^+) + K_a)}{d} \quad \text{(A4)}$$

$$\frac{[MnE_2]}{m} = \frac{[aK_a/((H^+) + K_a)]^2}{d} \quad \text{(A5)}$$

m is the total concentration of manganese species present, a is the total acetylacetone species present, and d is equal the sum of the three numerators in eq A3–A5. Equilibrium isotope effects are important in these calculations, but if they are neglected here for the purpose of this calculation and it is assumed that $(H_3O^+) \equiv (D_3O^+)$, then $[Mn^{2+}]/m = 0.798$, $[MnE^+]/m = 0.198$, and $[MnE_2]/m = 3.8 \times 10^{-3}$.

(11) Martell, A. E.; Smith, R. M. "Critical Stability Constants"; Plenum Press: New York, 1977; pp 245.

This holds as long as $m \ll a$.

Appendix II

Consider the equilibrium in eq A6, where HA is enol acetylacetone. If m equals the total Mn(II) present and a is equal to the total acetylacetone enol form species present, then $a = [HA] + [MnA^+] + [MnA_2]$ ($[A^-]$ is extremely small at the pH's used in this study) and $m = [Mn^{2+}] + [MnA^+] + [MnA_2]$. $[MnA_2]$ under the conditions of the experiments is small in comparison to $[Mn^{2+}]$ and $[MnA^+]$ and is neglected here. Then, if activity coefficients are assumed to be unity,

$$Mn^{2+} + HA \rightleftharpoons MnA^+ + H^+$$

$$K_{ass_1} = \frac{(MnA^+)(H^+)}{(Mn^{2+})(HA)} \qquad (A6)$$

$$K_{ass_1} = \frac{[MnA^+][H^+]}{(m - [MnA^+])(a - [MnA^+])}$$

and

$$am - (a + m + (H^+)/K_{ass_1})[MnA^+] + [MnA^+]^2 = 0$$

From Appendix I we see that $a \gg [MnA^+]$ and $\gg m$, at the highest m concentration used, and is at least 10 times the concentration of MnA^+. Therefore $[MnA^+]^2 \ll a[MnA^+]$ and

$$\frac{m}{a + m + (H^+)/K_{ass_1}} \simeq \frac{[MnA^+]}{a} \simeq P_{B_1}$$

where P_{B_1} is the mole fraction of the enol form of acetylacetone that is bound.

A similar treatment for association of the Mn(II) with the diketo form of acetylacetone, without proton loss, can be developed. Following the same lines, one can derive the parallel expression of eq A7, where P_{B_2} is the mole fraction of the diketo form of acetylacetone that is bound, k is the concentration of the diketone form, and K_{ass_2} is the association constant between Mn(II) and the diketone form. In this and in the previous corresponding expression for the mole fraction of the enol form it appears that the concentrations k and a in the denominator are negligible with respect to $1/K_{ass_2}$ and $(H^+)/K_{ass_1}$, respectively, since the slopes (Table II) are relatively independent of a substantial change in k or a.

$$P_{B_2} = \frac{[MnK^{2+}]}{k} \simeq \frac{m}{k + m + 1/K_{ass_2}} \qquad (A7)$$

Registry No. Acetylacetone, 123-54-6; acetylacetone enol, 1522-20-9; (*Z*)-maleylacetone, 40609-69-6; (*Z,E*)-maleylacetone enol, 77415-36-2; (*Z,Z*)-maleylacetone enol, 25568-65-4; 3,3-dimethyl-2,4-pentanedione, 3142-58-3; acetone, 67-64-1.

Conformational Analysis of Steroids: Polymorphic Forms of 17β-Acetoxy-6β-bromo-4-androsten-3-one

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X-ray crystal structure analysis of two polymorphic forms of 17β-acetoxy-6β-bromo-4-androsten-3-one provided three independent observations of the molecular conformation of this molecule. Polymorph I (mp 114–117 °C) was obtained by bromination of 17β-acetoxy-4-androsten-3-one with *N*-bromosuccimide and by acetylation of 6β-bromo-17β-hydroxy-4-androsten-3-one and consisted of conformers a and b in a 1:1 ratio. Polymorph II (mp 138–141 °C) was obtained by a treatment of polymorph I with chloroform–methanol (9:1) under epimerization condition and consists of one conformer only. Despite differences in solid IR spectra and dissimilarities in crystal packing environment, the three conformers are nearly identical in overall shape. The A rings of the three molecules have 1α-sofa conformations, and the B and C rings have chair conformations. The stacking of the 3-carbonyl groups in polymorph I contributes to denser packing and a shift in carbonyl frequency. The closest contact in the polymorph II involves the acetate carbonyl and is also reflected in a shift in spectra. The structure determinations demonstrate that while crystal packing has very little influence on overall molecular conformation, it does influence solid-state spectra.

Although the initial report[1] of synthesis of 6β-bromotestosterone acetate[2] described crystals of melting point 140–142 °C, repetition of the same procedure in our hands gave only crystals of melting point 114–117 °C. In the course of preparing 6-bromo-substituted androgens for use in affinity labeling of estrogen synthetase, dimorphic forms of 6β-bromotestosterone acetate were isolated.[3] The lower melting polymorph I was obtained by bromination of testosterone acetate and also by acetylation of 6β-bromotestosterone and was repeatedly recrystallized from 95% EtOH. The higher melting point polymorph II was obtained together with 6α-bromotestosterone acetate by epimerization treatment in $CHCl_3$–MeOH and was also repeatedly recrystallized from 95% EtOH. The integrity of each polymorph was maintained through recrystallization unless seeds of the alternate form were added to the solution, in which case the seed form was obtained. If the 95% ethanol solution of either polymorph was passed through a Millipore filter, subsequent recrystallization

(1) C. Djerassi, G. Rosenkranz, J. Romo, S. Kaufman, and J. Pataki, *J. Am. Chem. Soc.*, **72**, 4534 (1950).

(2) Trivial names and abbreviations used in this manuscript are as follows: NBS = *N*-bromosuccimide, testosterone = 17β-hydroxy-4-androsten-3-one, 6β-bromotestosterone = 6β-bromo-17β-hydroxy-4-androsten-3-one, 6β-bromotestosterone acetate = 17β-acetoxy-6β-bromo-4-androsten-3-one.

(3) M. Numazawa and Y. Osawa, *Steroids*, **34**, 347 (1979).

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