Kinetic Studies of Fast Equilibrium by Means of High-performance Liquid Chromatography. Part 11.[1] Keto–Enol Tautomerism of Some β-Dicarbonyl Compounds

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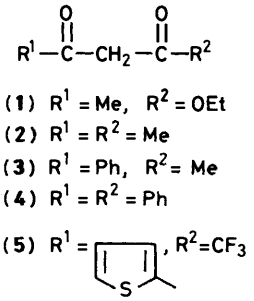
The keto–enol tautomerism of some β-dicarbonyl compounds, including ethyl acetoacetate (**1**), acetylacetone (**2**), benzoylacetone (**3**), dibenzoylmethane (**4**), and 1,1,1-trifluoro-3-(2-thenoyl)acetone (**5**), has been investigated in various solvents at 25 °C, by using low-temperature (−20 to −50 °C) high-performance liquid chromatography (h.p.l.c.) for analytical measurements. Base-line separation of keto and enol tautomers of these compounds has been achieved on silica gel packings with a mobile phase composed of hexane and propan-1-ol containing a small amount of acetic acid. The ratios of keto and enol tautomers in different solvents have been determined; the percentages of enol tautomers have been found to be higher in polar solvents, in accord with n.m.r. data previously reported. In all solvents the proportions of enol tautomers of acetylacetone derivatives have been found to increase in the following order; (**2**) < (**3**) < (**4**); this has been attributed to the fact that enol tautomers of phenyl-substituted acetylacetones have been stabilized, owing to the formation of extended conjugated systems. For the ester (**1**), the presence of a very small amount of a third tautomer (0.1%) has been found, probably an unconjugated enol tautomer.

In this series of papers a method has been described for investigating fast equilibrium by means of high-performance liquid chromatography (h.p.l.c.) based on conventional principles. When h.p.l.c. is carried out at low temperatures, labile species present in the equilibrium state may be detected without any change during chromatography, because the reaction rates decrease with the fall in temperature while the separation speed is still fast. We have already successfully separated labile metal chelates,[2,3] rotational isomers,[1,4,5] and sugar anomers.[6] In the h.p.l.c. procedure, when base-line separation is achieved, peak heights (or areas) appearing on chromatograms directly indicate the equilibrium concentrations of each species in the state prior to h.p.l.c. We have also demonstrated that determination of rate constants is possible.[2,4,5,7,8] In a preliminary communication,[9] we have shown that keto–enol tautomerism can also be studied by h.p.l.c. The enolization reaction is probably the most well known example of prototropic tautomerism, and is closely linked with various reactions of carbonyl compounds such as halogenation, deuteriation, and racemization. Since the description of Meyer's bromine titration method,[10] keto–enol tautomerism has been extensively studied by many workers.[11] Spectroscopic methods such as u.v., i.r., and especially n.m.r. have facilitated the determination of the ratios of tautomers of various carbonyl compounds, including β-dicarbonyl derivatives. The present report deals with an h.p.l.c. study of keto–enol tautomerism of five β-dicarbonyl compounds.



Experimental

Reagents.—The following commercially available β-dicarbonyl compounds, considered to be of the highest grade, were used: ethyl acetoacetate (**1**), pentane-2,4-dione (acetylacetone) (**2**), 1-phenylbutane-1,3-dione (benzoylacetone) (**3**), 1,3-diphenylpropane-1,3-dione (dibenzoylmethane) (**4**), and 4,4,4-trifluoro-1-(2-thienyl)butane-1,3-dione [1,1,1-trifluoro-3-(2-thenoyl)acetone] (**5**). These were purified either by distillation [for (**1**) and (**2**)] or recrystallization from chloroform–hexane [for (**3**)—(**5**)].

Apparatus.—In order to operate h.p.l.c. at low temperatures, column and flow paths, including an injector sample loop, were immersed in a bath thermostatically controlled at low temperature (25 to −70 °C) (Figure 1). A sample solution at room temperature (25 °C) was sucked into the cooled injector loop. Thus, the sample solution was cooled rapidly and then moved to the column head prior to the separation process. H.p.l.c. was found possible even at −70 °C on silica gel packings if a suitable low melting eluant was used. After the separation of keto and enol tautomers, column effluent was passed through a long, narrow, stainless steel tube (0.5 mm int. diam., 10 m length) thermostatically maintained at high temperature in an air-bath. This heating of the column effluent caused keto–enol equilibration before the effluent reached the cell of the u.v. detector. As a result, the ratios of the two peak areas attained a constant value, and from measurements of the peak areas the population of each tautomer prior to h.p.l.c. were directly obtained. This preheating treatment is indispensible for the determination of tautomer ratio because the absorption coefficients of the conjugated enol forms are sometimes more than 100-fold larger than those of the unconjugated keto forms.

Results and Discussion

Ethyl Acetoacetate (**1**).—The interconversion between keto and enol tautomers in the pure state is not rapid; however, the presence of a minute amount of H^+ or especially OH^- promotes the interconversion catalytically.[12] Thus, reverse-phase chromatography in protic aqueous solvents is not suitable for separation. We therefore tried adsorption

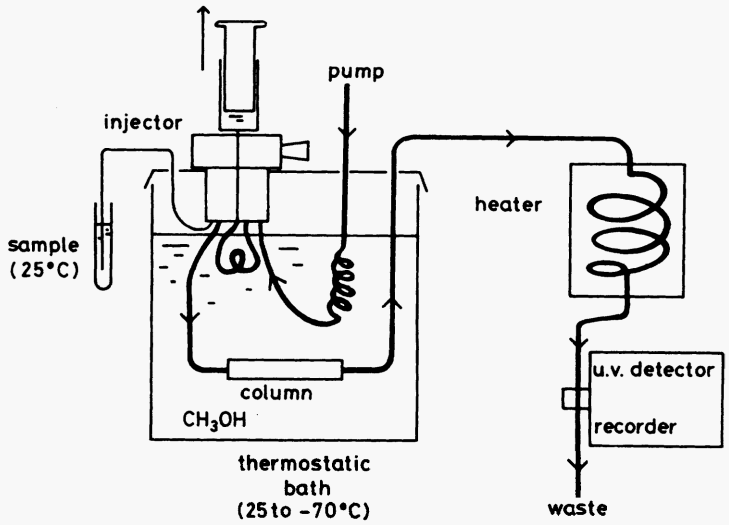


Figure 1. H.p.l.c. apparatus for low-temperature measurements

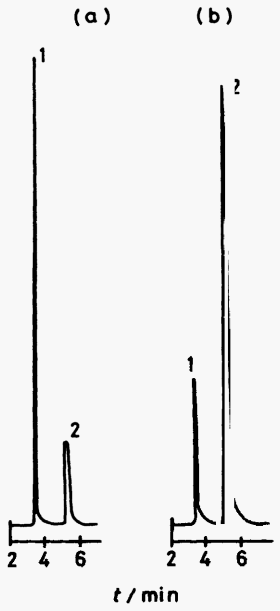


Figure 2. H.p.l.c. of (1) at −40 °C with and without re-equilibration (for chromatographic conditions, see Figure 3): (a) without re-equilibration, (b) heated at 90 °C for 1 min; 1, enol form; 2, keto form

chromatography on silica gel packings, in spite of the weak acidity of the silanol groups (other, milder packings were not available). The mixed solvent system of hexane and propan-1-ol containing a small amount of acetic acid gave satisfactory results. The addition of acid to the eluant is not favoured, but very broad, tailing peaks were obtained in its absence. Since the present solvent system is not very polar, the concentration of H^+ and (especially) OH^- is low. Separation of the keto and enol tautomers of the ester (1) was achieved at −20 °C.[9] If neutral packings milder than silica gel become available, with non-polar solvents in the absence of acid, separation of keto and enol tautomers may be attained at higher column temperatures. We also examined the chromatographic behaviour of the ester (1) at lower temperatures. When h.p.l.c. was carried out at −40 °C and the column effluent was introduced immediately into the cell of the u.v. detector without heating, chromatograms of the type shown in Figure 2(a) were obtained. On the other hand, after preheating, quite different chromatograms were obtained [Figure 2(b)]. This is a result of the difference in absorption coefficients of conjugated enol form and unconjugated keto form. In order to find the optimum conditions for post-column heating for re-equilibration in the eluant used, the temperature of the heating bath was varied from 30 to 150 °C. When the temperature was higher than 90 °C, the ratios of the two peak areas became constant (flow rate 2.5 cm^3 min^{-1}, column temperature −45 °C). When the heating temperature was less than 90 °C, the proportion of the peak with shorter retention time in Figure 2 increased gradually with the fall in temperature; this suggests that 90 °C is necessary for re-equilibration. When the flow path length was shorter than 10 m, a higher heating temperature was necessary, and *vice versa*. The ratios of two tautomers could be calculated directly, if re-equilibration in the effluent was attained. The small peak in Figure 2(b) (*ca.* 8% of the total) is attributed to the enol tautomer by comparing the h.p.l.c. results with n.m.r. data.[11]

Besides these two large peaks, a new very small peak [peak 3 in Figure 3(f)] appeared and was separated at −60 °C. When portions corresponding to peak 3 were collected and then re-chromatographed, the same chromatogram as Figure 3(f) was obtained again. Thus, this minor peak cannot be attributed to an impurity, but must be due to an isomeric species existing in the equilibrium state. The area of peak 3 in Figure 3(f) amounts only to *ca.* 0.1% of the total. The following three possibilities exist for the identity of this minor component (since enolization of unsymmetrical β-dicarbonyl compounds may occur in various ways).

(*a*) The enol form of a β-dicarbonyl compound usually exists as the conjugated *cis*-enol, stabilized by intramolecular hydrogen bonding. Two different isomeric *cis*-enols are distinguishable in cases of unsymmetrical β-dicarbonyl compounds [reaction (i)]; interconversion occurs by transfer of an enol proton from one oxygen atom to the other. This has little effect on the shape of the molecule. N.m.r. studies previously reported failed to distinguish these two enols, though u.v. and i.r. studies have sometimes distinguished them.[11,13] From linewidth measurements in the ^{17}O n.m.r. spectra of β-diketones, the lower limit of the rate constants for the interconversion between two *cis*-enols is found to be at least 10^5—10^6 s^{-1} at room temperature.[14] This eliminates the possibility that two *cis*-enols could be separated

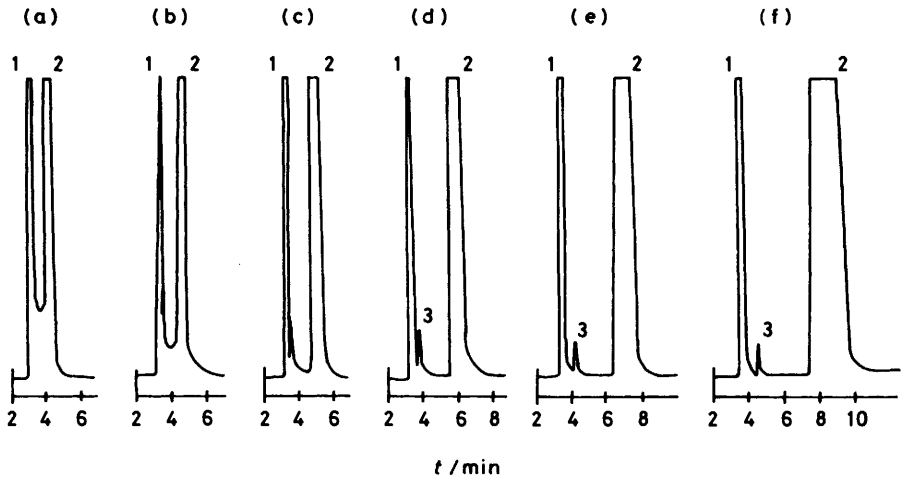
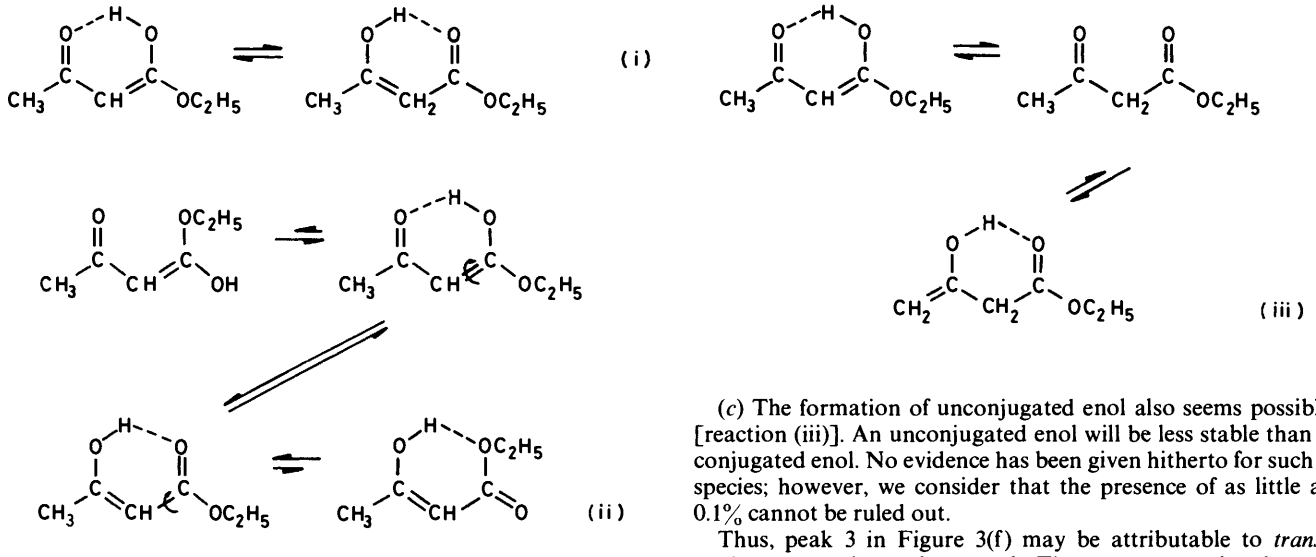


Figure 3. H.p.l.c. of (**1**) at low temperatures; column Polygosil 60—5 (4.6 mm × 15 cm); eluant hexane–propan-1-ol–acetic acid (100:10:3), 2.5 cm^3 min^{-1}; detector u.v. 270 nm; sample pure liquid (2.5 × 10^{-3} cm^3); column temperature (a) −18 °C, (b) −30 °C, (c) −40 °C, (d) −50 °C, (e) −55 °C, (f) −60 °C; 1, enol form; 2, keto form; 3, see text



by h.p.l.c., because the rate constants for interconversion are estimated to be 10^2 s^{-1} or larger, even at −60 °C, if we assume that the reaction rates are tripled by a 10 °C rise in temperature.

(*b*) The formation of *trans*-enol may occur. Under the usual conditions, *cis*-enol is more stable than *trans*-enol by virtue of intramolecular hydrogen bonding. The *trans*-enol, however, may be stabilized to some extent by solute–solvent intermolecular hydrogen bonding in polar protic solvents. The presence of *trans*-enol is still controversial. Several authors have claimed to detect a substantial amount of *trans*-enol.[15] Other authors,[16] however, have denied the presence of *trans*-enol and consider that the presence of a small amount of impurity contaminating the synthetic procedure has led to an erroneous conclusion. The interconversion between *cis*- and *trans*-enol proceeds *via* intramolecular rotation about a carbon–carbon bond, which has partial double-bond character due to conjugation [reaction (ii)]. We have reported previously[1,4,5] that rotamers about a partial double bond can be separated by h.p.l.c. at low temperatures. Thus, it is not unlikely that a minute amount of *trans*-enol is present and separated by h.p.l.c. at low temperatures.

(*c*) The formation of unconjugated enol also seems possible [reaction (iii)]. An unconjugated enol will be less stable than a conjugated enol. No evidence has been given hitherto for such a species; however, we consider that the presence of as little as 0.1% cannot be ruled out.

Thus, peak 3 in Figure 3(f) may be attributable to *trans*-enol or unconjugated *cis*-enol. The present results do not indicate decisively which explanation is correct. Other spectral methods such as n.m.r. could not be used owing to the very small proportion of the minor component. We consider, however, that the latter explanation is the more logical, for the following reason. When h.p.l.c. was carried out at −60 °C, and the column effluent was passed through the cell of the u.v. detector immediately, without heating, peak 3 was small. This suggests that the absorption coefficient of this minor component is small, as expected for unconjugated enol.

β-*Dicarbonyl derivatives* (**2**)—(**5**).—The presence of both keto and enol tautomers for (**2**), (**3**), and (**5**) had been previously confirmed by various methods such as u.v., i.r., and n.m.r.; the ratios of keto and enol tautomers had been determined in particular by n.m.r.[1,17] On the other hand, compound (**4**) is almost completely enolized; the presence of the keto form was not evident from ^1H n.m.r.[18] or ^{13}C n.m.r.[19] H.p.l.c. of these acetylacetone derivatives was carried out at various column temperatures. Separation of keto and enol tautomers for these compounds, including (**4**), was achieved at lower column temperature than for (**1**) (−40 to −50 °C). The discrepancy between previous n.m.r. data and the present h.p.l.c. results for

Table. Equilibrium constants (K = [enol]/[keto]) for keto–enol tautomerism of β-dicarbonyl compounds (**1**)—(**5**) in various solvents at 25 °C

	(1)			(2)			(3)		(4)		(5)	
	This work			This work			This work		This work		This work	
	0.01M	0.1M	N.m.r.[a]	0.01M	0.1M	N.m.r.[a]	0.01M	0.1M	0.01M	0.1M	0.01M	0.1M
Hexane	1.07	0.94	0.64	48	31	19	71	68	98		17	
Cyclohexane	1.13	0.98		42	26		74	70	89		20	
Carbon tetrachloride	0.80	0.68	0.39	23	24	24	41	73	44	46	15	13
Chloroform	0.29	0.15	0.081	6.0	5.3	6.7	14	36	24	23	10.1	9.7
Dichloromethane	0.23	0.14		4.1	2.6		11	12	23	21	6.6	7.1
Ethyl acetate	0.27	0.14		5.9	4.0		15	15	36	38	6.4	6.1
Diethyl ether	0.54	0.50	0.29	18	9.1	19	34	34	30	58	7.5	8.8
1,4-Dioxane	0.19	0.18	0.12	3.4	4.6	4.6	12	12	19	23	4.7	3.9
Tetrahydrofuran	0.33	0.25		6.5	4.3		18	13	23	32	0.59	0.69
Acetonitrile	0.062	0.085	0.052	2.2	1.7	1.6	4.4	4.8	12	11	3.3	4.1
Acetic acid	0.099	0.089	0.019	3.5	2.7	2.0	17	8.3	20	21	7.4	13
Ethanol	0.18	0.16	0.11	6.0	3.8	4.6	14	14	26	32	See text	
Methanol	0.12	0.079	0.062	3.3	2.6	2.8	9.5	8.0	24	16	See text	
Water	0.057	0.053		0.34	0.34							
Pure liquid	0.092		0.081	3.9		4.3						

[a] M. T. Rogers and J. L. Burdet, *Can. J. Chem.*, 1965, **43**, 1516. These are values for 0.1 molar ratio (much more concentrated than this work).

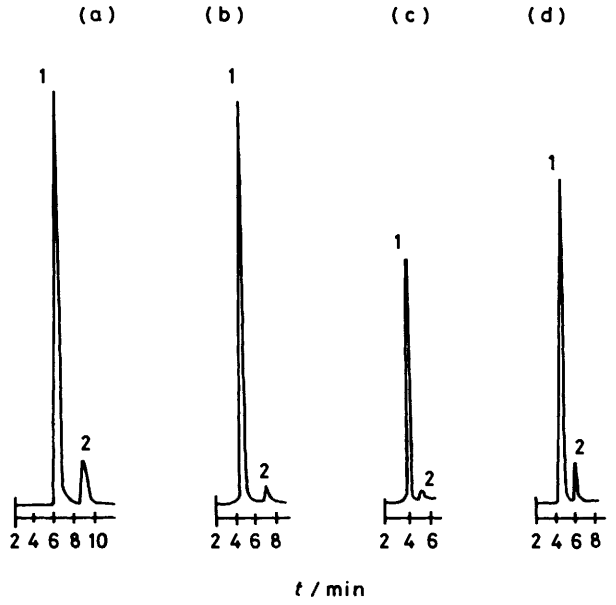


Figure 4. Separation of keto and enol tautomers of (**2**)—(**5**); column Polygosil 60—5 (4.6 mm × 15 cm) [for (b), (c), and (d)] and LiChrosorb SI 1 000 (10 μm, 4.0 mm × 25 cm) [for (a)]; eluant hexane–propan-1-ol–acetic acid (100:10:3), 2.5 cm^3 min^{-1}; detection u.v. 270 nm; sample, each 0.5% $CHCl_3$ solution, 2.5 × 10^{-3} cm^3; column temperature (a) −50 °C, (b) −40 °C, (c) −40 °C, (d) −40 °C; (a) (**2**), (b) (**3**), (c) (**4**), (d) (**5**); 1, enol form, 2, keto form

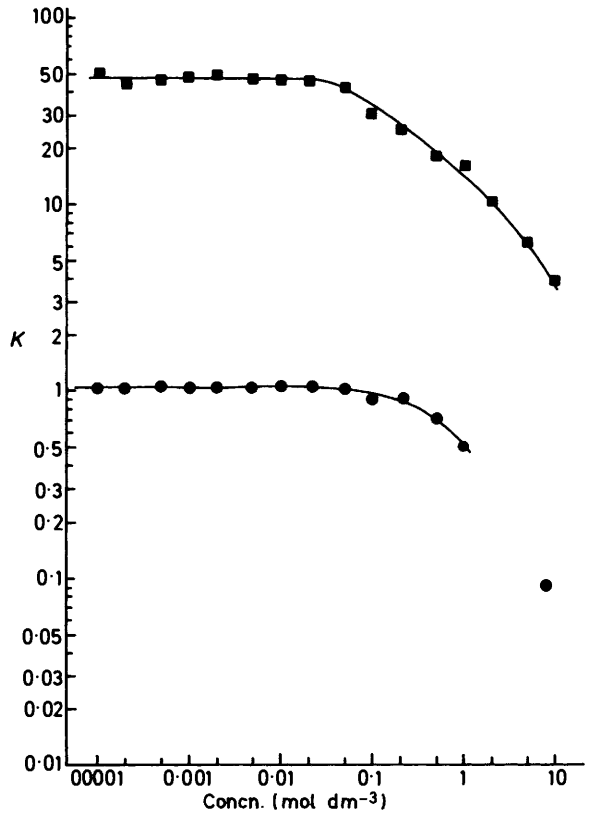


Figure 5. Dependence of equilibrium constants of keto–enol tautomerism (K = [enol]/[keto]) for (**1**) (●) and (**2**) (■) in hexane on concentration

(**4**) may be interpreted in terms of better detectability by h.p.l.c. The present results suggest that h.p.l.c. is also a powerful tool for investigating fast equilibria, and may even be better than n.m.r. when the content of the minor component is very low. Figure 4 indicates the separation of these acetylacetone derivatives under optimum h.p.l.c. conditions. Under these conditions enol tautomers, including (**4**), were eluted faster than keto tautomers. This seems surprising since a hydroxy group is adsorbed on the surface of silica gel more strongly than a carbonyl group. However, *cis*-enols form strong intramolecular hydrogen bonds, which will be disturbed by adsorption, whereas with keto tautomers no intramolecular hydrogen bonding exists. In contrast to (**1**), no third peak was detected for these acetylacetone derivatives.

Ratios of Keto and Enol Tautomers.—When h.p.l.c. is carried out at low temperature, and base-line separation is attained, the chromatograms indicate directly the equilibrium concentrations

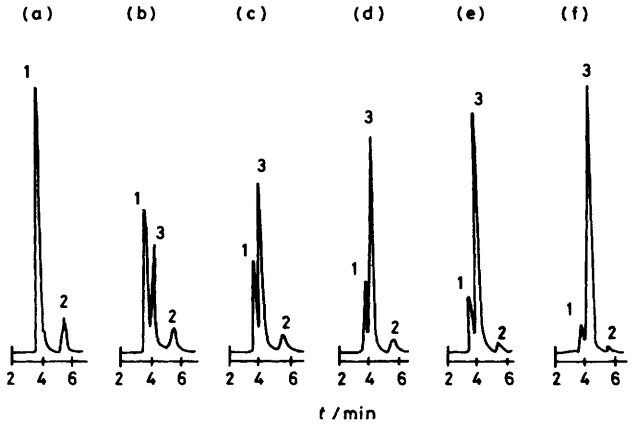
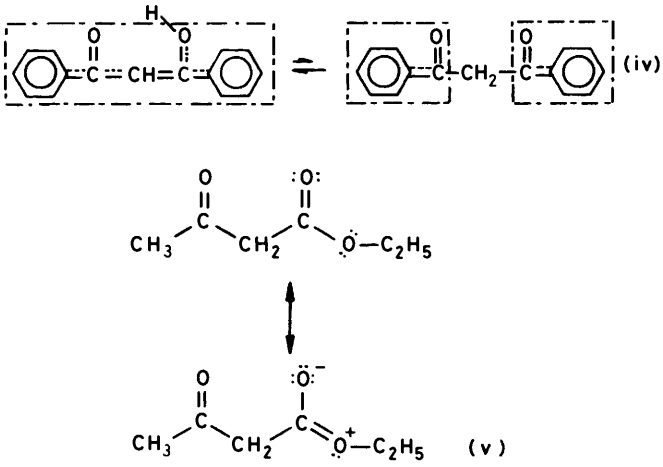


Figure 6. Hemiacetal formation from (**5**) in methanol (for chromatographic conditions, see Figure 4); sample 0.5% methanolic solution of (**5**), 2.5 × 10^{-3} cm^3; (a) after 0.5 min, (b) 6 min, (c) 12 min, (d) 18 min, (e) 24 min, (f) 60 min (equilibrium)



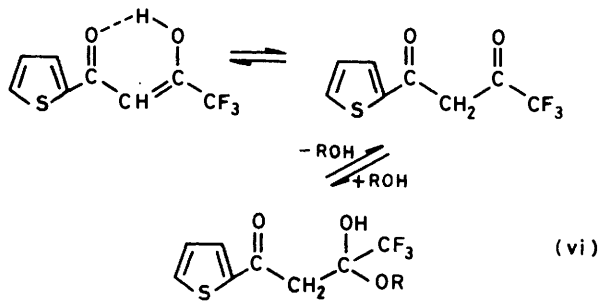
of each tautomer prior to h.p.l.c. so long as post-column heating attains the re-equilibration completely. Thus, samples dissolved under different conditions (solvent composition, concentration, and temperature) give different chromatograms. The five β-dicarbonyl compounds were dissolved in a variety of solvents at 25 °C, and after equilibration were analysed by h.p.l.c. at low temperature. After separation of keto and enol tautomers the column effluent was heated at 100 °C [for (**1**)] or 60 °C [for (**2**)—(**5**)] for about 1 min, for re-equilibration. The equilibrium proportions of enol tautomer were thus determined at two different concentrations (0.1 and 0.01 mol dm^{-3}) from peak area measurements; the results are summarized in the Table. When the column temperature was changed in the low-temperature region, the ratios of the two tautomers were unchanged so long as base-line separation was attained. Similarly, the ratios were not affected by change in composition of the eluant (*i.e.* the content of propan-1-ol and acetic acid). The following observations are noteworthy.

(*a*) The ratios are sensitive to solvent composition: the proportion of enol form is higher in non-polar solvents. This point has already been extensively discussed.[20] The present h.p.l.c. results agree fairly well with n.m.r. data previously reported.[11,17]

(*b*) The ratios are also sensitive to concentration: the proportion of enol form is usually higher when the concentration is low. Figure 5 indicates the proportion of enol form for (**1**) and (**2**) in hexane at different concentrations.

(*c*) The following sequence of increasing percentages of enol tautomers was observed in all solvents; (**1**) < (**2**) < (**3**) < (**4**). This is explained in terms of the formation of the extended conjugation system. The enol tautomer of (**4**) forms a highly conjugated system which extends over the whole molecule, but is broken for keto form [reaction (iv)]. The small percentage of the enol form of (**1**) is explained in terms of resonance (v), which will disturb the formation of the conjugated enol form to some extent.

(*d*) Some characteristic effects of individual solvents seem to exist. For example, in tetrahydrofuran the keto form content of (**5**) is very high.



Hemiacetal Formation of (**5**).—When alcoholic solutions of (**5**) were chromatographed, quite different chromatograms were obtained (Figure 6). When h.p.l.c. was carried out immediately after dissolution, two peaks corresponding to keto and enol tautomers appeared. When the sample was left for some time and chromatographed repeatedly at intervals, a new peak appeared which grew gradually. After 40 min, the new peak had reached a maximum. These observations suggest that some reaction took place and reached equilibrium; this is attributed to the hemiacetal formation.[21] Since attempts to isolate the hemiacetal of (**5**) were unsuccessful,[21] it appears that a labile equilibrium is present in solution. Figure 6 suggests that about 90% of (**5**) exists as hemiacetal in methanol. Similar chromatograms were obtained in ethanol solution; in this case about 60% of (**5**) was transformed into the hemiacetal. Hemiacetal formation was not observed for the other β-dicarbonyl compounds tested. The strongly electron-withdrawing fluorine atoms presumably promote hemiacetal formation [reaction (vi)]. The present results show that hemiacetal formation can be traced by h.p.l.c.

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