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KINETIC STUDIES OF FAST EQUILIBRIUM BY MEANS OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

XV*. SEPARATION OF TAUTOMERS OF β-DIKETOESTERS

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SUMMARY

Keto–enol tautomerism of sixteen β-diketoesters, including cyclic ones, has been investigated using low-temperature high-performance liquid chromatography (HPLC) for analytical measurements. Keto–enol tautomers of these β-diketoesters have been successfully separated on chemically bonded silica gel packings which contain hydroxyl-groups (diol-type column packings). These phases gave much better results than the usual silica gel packings. Base-line separation of keto- and enol-tautomers of the β-diketoesters has been achieved on diol-type column packings with a mobile phase consisting of hexane and a small amount of 1-propanol and at a higher column temperature than on silica gel packings. The interconversion between tautomers of 2-alkyl-substituted β-diketoesters is so slow that keto- and enol-tautoesters have been separated even at 25°C on diol-type column packings. The ratios of keto- and enol-tautomers have been determined by HPLC in different solvents, and showed good agreement with data previously reported. The presence of other species in equilibrium, such as hemiacetals and possibly unconjugated enol-tautomers has been observed for some of the β-diketoesters tested.

INTRODUCTION

In this series of papers[1–8] we have demonstrated that labile species, which are in equilibrium in solution, can be separated by means of high-performance liquid chromatography (HPLC) at low column temperatures without any interconversion during chromatography, because this is slowed down by the decrease in temperature. Our previous report[7] has indicated that the separation of tautomers is possible by this technique. The present report deals with the separation of tautomers of sixteen β-diketoesters, including alicyclic ones, by chromatography on diol-type column packings.

* For part XIV see ref. 1.

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EXPERIMENTAL

The following sixteen β-diketoesters were obtained commercially: (a) methyl acetoacetate, (b) ethyl acetoacetate, (c) propyl acetoacetate, (d) butyl acetoacetate, (e) isobutyl acetoacetate, (f) *sec.*-butyl acetoacetate, (g) *tert.*-butyl acetoacetate, (h) pentyl acetoacetate, (i) isopentyl acetoacetate, (j) benzyl acetoacetate, (k) ethyl 2-methyl-acetoacetate, (l) ethyl 2-ethylacetoacetate, (m) ethyl 4-chloroacetoacetate, (n) ethyl benzoylacetate, (o) ethyl 2-oxo-1-cyclopentanecarboxylate and (p) 2-oxo-1-cyclohexanecarboxylate. These β-diketoesters were distilled before use if necessary, but it was sometimes impossible to remove minute amounts of impurities by distillation. When a small peak appears in HPLC chromatograms, it is necessary to investigate whether this small peak should be attributed to an impurity or to a minor tautomer, which is part of the equilibrium. This is easily carried out by collecting the relevent fraction of the chromatogram and re-chromatographing it. If the small peak is due to an impurity, an unchanged chromatogram will be obtained. On the other hand, if the small peak is attributable to a labile isomeric species, the repeated re-chromatography will give changing patterns, and finally, equilibrium concentrations will be observed in the chromatograms.

The HPLC apparatus for low-temperature measurements used in our previous work was modified (Fig. 1). In order to operate HPLC at low temperatures, the column, connecting capillaries, and, if necessary, the sample loop of the injector were immersed in a bath thermostated at a low temperature (bath cooler: Neocool Bath, Model BD-51, Yamato Scientific Co., temperature controller: Model SR-31, Yamato Scientific Co.). Before elution, the HPLC solvent was thoroughly cooled by passing it through a cooled coiled stainless-steel tube (or a small empty column). A sample solution at room temperature (25°C) was sucked into the injector loop. Thus, the sample solution was cooled rapidly prior to the separation process and then injected on the top of the column. After the separation of keto and enol tautomers, the column effluent was passed through a long narrow stainless-steel tube (10 m × 0.5 mm I.D.), thermostated at higher temperatures (80–150°C) in an air bath (Model ASB-200, Japan Spectroscopic Co.). This heating of the column effluent caused keto–enol equilibration before the effluent reached the cell of the UV detector. As a result, the ratios of the two peak areas reached a constant value, and from measure-

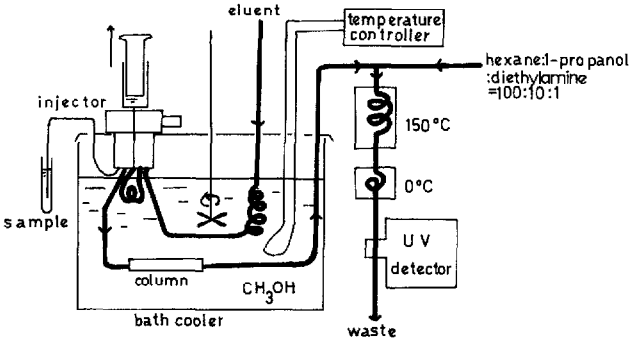


Fig. 1. HPLC apparatus for low-temperature measurements.

ments of the peak areas the population ratios of each tautomer prior to HPLC were directly obtained. This heat treatment is indispensable for the determination of tautomer ratios, because the absorption coefficients of the conjugated enol forms are sometimes more than 100-fold larger than those of the unconjugated keto forms. However, the heat treatment, was found to yield incomplete equilibration in a few cases, because the interconversion between tautomers of a few β-diketoesters, especially 2-alkyl-substituted ones, was very slow. In such cases, the addition of a small amount of a base such as diethylamine to the column effluent before heating was found to be very effective. It is a well-known fact that the rate of interconversion between keto- and enol-tautomers is drastically increased in the presence of a small amount of a basic substance. With hexane containing a small amount of 1-propanol as the solvent, HPLC was found to be possible even at -70°C on silica gel or diol-type column packings, A slight, gradual loss of column efficiency was observed with decreasing temperature.

Common silica gel packings (LiChrosorb SI-100, 10 μm or LiChrosorb SI-60, 10 μm, from Merck, Darmstadt, F.R.G., and Polygosil 60-5, 5 μm, Macherey-Nagel, Düren, F.R.G. and diol-type column packings (LiChrosorb DIOL, 10 μm, from Merck) were slurry packed into stainless-steel column (15 cm $\times$ 4.6 mm I.D.). The mobile phase consisted of hexane and 1-propanol in different ratios: A, 100:6; B, 100:4; C, 100:2; D, 100:1.5. The column temperatures at which complete separation of tautomers could be obtained differed for various β-diketoesters. The required temperature was coded as follows: I, -15°C; II, -10°C; III, -5°C; IV, 25°C.

RESULTS AND DISCUSSION

Comparison of silica gel and diol-type column packings

In previous work[7] we have shown that the separation of keto- and enol-tautomers of ethyl acetoacetate is feasible on silica gel packings with mixed solvents consisting of hexane, 1-propanol, and acetic acid. For the base-line separation of tautomers, sub-ambient column temperatures (< -20°C) are necessary. However, the above combination of stationary and mobile phase is not always suitable for the separation of tautomers for the following reasons: (1) The presence of acetic acid in the eluent is not desirable, because both H^+ and OH^- ions have a catalytic effect on the interconversion between keto- and enol-tautomers. However, the presence of acetic acid is indispensable for the separation of tautomers on silica gel packings, because otherwise broad and tailing peaks are always obtained. (2) Silica gel is apparently not such a favourable column packing due to the relatively strong acidic properties of the silanol groups. It is highly likely that interconversion is accelerated on the surface of the silica gel. Thus, less active and neutral column packings are more desirable. We therefore attempted the separation of tautomers on diol-type column packings. In comparison to silica gel packings, the alcoholic OH groups on diol phases are less acidic. Sharp peaks were obtained on diol-type column packings with the solvent system hexane–1-propanol in the absence of acetic acid. Base-line separation of tautomers of ethyl acetoacetate was possible at -10°C, which is about 15°C higher than on silica gel packings.

Separations of tautomers on diol-type packings

The separation of the sixteen β-diketoesters was attempted on diol-type column packings with a solvent system containing hexane and a small amount of 1-propanol (1.5–6%). The separation of keto- and enol-tautomers of these β-diketoesters was found to be possible at temperatures that were about 15–20°C higher than those required for silica gel packings. Fig. 2 shows an example of the separation of tautomers of a few β-diketoesters. For pure sample f, the peak area of the former (small) peak (peak 1) in Fig. 2B is about 10% of the total, which is in agreement with the abundance of the enol-form of pure f[10]. On the other hand when a dilute solution of f in hexane was chromatographed, the peak area of 1 increased to half the total area, in accordance with the observation[10] that in dilute hexane solutions the percentages of the enol-form of various β-diketoesters are close to or larger than 50. Therefore, peak 1 in Fig. 2B was attributed to the enol-form and peak 2 to the keto-form. With similar procedures the peak identification of other β-diketoesters was carried out[9–12]. In all cases, enol-tautomers were eluted faster than keto-tautomers, because enol-tautomers are less polar than keto-tautomers by dint of their strong ability to form chelated intramolecular hydrogen bonds. In most cases, base-line separation of tautomers was possible at a column temperature of −10 to 0°C. Among the β-diketoesters tested, the interconversion was found to be very slow for 2-alkyl-substituted compounds. The separation of the keto- and enol-forms of k and l was found to be possible at 25°C on diol-type column packings, whereas on silica-gel packings the separation was impossible, even at 0°C.

Ratios of keto- and enol-tautomers

When base-line separation of tautomers was achieved, the ratios of tautomers in the sample prior to HPLC could be directly calculated from peak area measurements, provided that the post-column heating brought about the equilibration of tautomers in the column effluent. In order to fulfill this condition, rather high heating

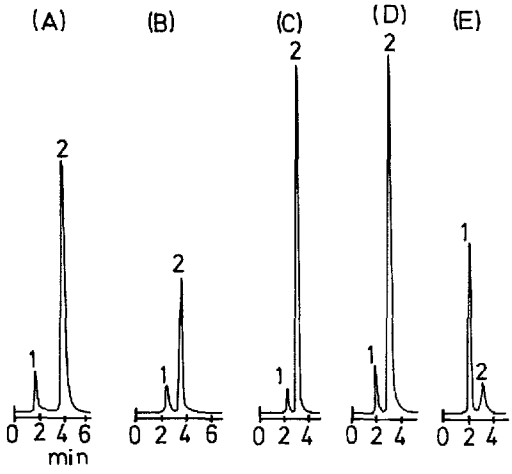


Fig. 2. Separation of keto(2)- and enol(1)-tautomers of β-diketoesters. Column: LiChrosorb DIOL (10 μm: 15 cm × 4.6 mm I.D.); sample identification: (A) a, (B) f, (C) k, (D) o, (E) p; flow-rate, 2.0 ml/min, UV 290 nm. For other HPLC conditions, see Table I.

TABLE I

PERCENTAGES OF ENOL-FORM OF β-DIKETOESTERS IN DIFFERENT SOLVENTS

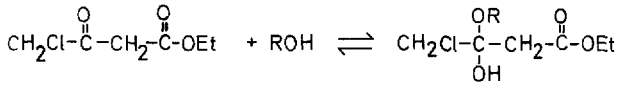
Compounds	*HPLC conditions*	*This work*				*Other works*		
		0.5% hexane	*0.5% chloroform*	*0.5% ethanol*	*Pure liquid*	*Dilute hexane*	*Dilute ethanol*	*Pure liquid*
a	A, I	57	13	16	6			5§
b	B, II	51	10	13	8	52*		8*, 8**
c	B, II	54	10	14	12			
d	C, II	54	10	14	15			15**
e	C, II	55	11	15	14			
f	C, II	51	9	15	10	51*		10**
g	C, II	45	8	19	10	46*		9*, 17**
h	D, III	54	10	15	19	52*		12*
i	D, III	55	10	14	17			
j	B, II	54	11	14	16			
k	C, IV	17	3	3	5			5**
l	C, IV	3	2	2	2			1**
m	B, II	44	20	Hemiacetal formation	15			
n	B, II	73	18	29	20			22**
o	C, I	35	4	8	5		5***	
p	C, I	89	80	83	74		57***	

* Ref. 10.
** Ref. 12.
*** Ref. 11.
§ Ref. 9.

temperatures (150°C) and the addition of a basic compound were necessary in a few cases, as described in the experimental section. The ratios of tautomers of the β-diketoesters in low concentration in three different solvents (ethanol, chloroform, and hexane) (0.5%) and in the pure state were determined. The results are shown in Table I. In dilute solutions, the ratios were almost independent of the concentration, but were affected by the kind of solvent used. This suggests that solute–solute interactions are much weaker than solute–solvent interactions at low concentrations. Thus, the values in Table I represent the ratios at very low concentrations. Ratios reported by other authors obtained by different methods[10–12] are also shown in Table I. They agree fairly with our HPLC results.

The following facts are noteworthy. (1) The concentrations of enol-tautomers are higher in the non-polar solvent hexane than in polar solvents such as ethanol and chloroform. For alkylacetoacetate (components a–j) the percentages of enol-tautomers are 50% or more, although under the usual conditions, the enol content of β-diketoesters is known to be low. (2) The percentages of enol-tautomers are low for the 2-alkyl-substituted acetoacetates, k and l. Since the separation of the tautomers of k and l is possible even at room temperature, the interconversion between the tautomers is remarkedly slow. (3) The cyclic β-diketoesters o and p show a charac-

teristic behaviour. The former is highly ketonic, and the percentage of enol-tautomer amounts to only 5–35% under various conditions. In contrast, the latter is highly enolic, as shown in Table I. This observation has already been discussed by other authors[11,14] and has been attributed to the fact that exo double bonds stabilize a 5-membered ring and destabilize a 6-membered ring. (4) For the non-substituted alkylacetoacetates a–j, a third small peak appeared which could be separated below −25°C. This has been observed earlier for b[7]. Although the relative area of the small peak was low, it was not attributed to an impurity. This was confirmed by re-chromatography. The small peak may be attributable to unconjugated enol[7]. (5) An ethanol solution of m gave another new peak. This peak was also observed when m was dissolved in other alcohols, such as methanol and 1-propanol, but was never observed in other solvents, such as hexane and chloroform. The retention time of this peak differed when the sample was dissolved in different alcohols, which suggested that this peak might be attributed to the products of a reaction of m with the alcohol. A similar phenomenon has been observed earlier for fluoro-substituted β-diketoesters[7,13]. The present results may be interpreted in terms of hemiacetal formation,



because strongly electron withdrawing halogen atoms decrease the electron density on carbonyl oxygen and consequently may promote hemiacetal formation.

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