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HPLC SEPARATION OF KETO AND ENOL TAUTOMERS OF SEVERAL KETONES AT LOW TEMPERATURES[1)]

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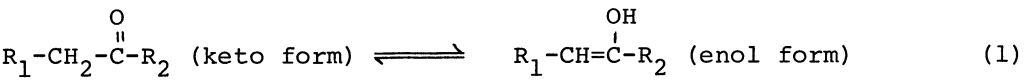
High-performance liquid chromatography of some ketones at -20 —— -60 °C achieved the separation of keto and enol tautomers. The ratios of keto and enol forms in different conditions were determined for β-diketones. Cyclohexanone also gave a small peak which was possibly attributed to enol tautomer along with a large peak of keto tautomer.

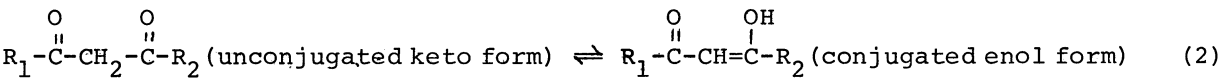
In our previous reports[2-4)] a method has been devised to investigate fast equilibrium by means of high-performance liquid chromatography(HPLC). When HPLC is operated at low temperatures, interconversion between dynamic isomers can be retarded effectively, and labile species can be separated without interconversion during chromatography. There exist numerous substances which are present as the equilibrated mixture of two or more dynamic isomers in solution. We have already reported the separation of rotamers[3,4)] and sugar anomers.[5)] The present report deals with the separation of keto and enol tautomers of ketones.

It has been well known that ketones and aldehydes may exist as the equilibrated mixture of two isomers (keto and enol form) in solution as shown below.



(1)

Simple ketones and aldehydes exist as keto form and the content of enol form is very low. Contrary to this, the ratios of enol tautomer are high in β-diketones since methylene groups of β-diketones are highly activated by two carbonyl groups and enol form has a conjugated structure.



(2)

In order to determine the ratios of keto and enol tautomers, slight modification of the apparatus[3,4)] was made; column effluent was passed through a long narrow stainless steel tube(0.5 mm in diameter and 10-20 m in length) which was heated at 60-90 °C in an air bath(Model ASB-200, Japan Spectroscopic Co., Ltd.). Thus, keto-enol tautomerism is re-equilibrated in the effluent. This treatment is indispensable for determining the ratios of keto and enol tautomers because absorption coefficients of cunjugated enol tautomers are sometimes more than two orders larger than those of unconjugated keto tautomers.

Ethyl acetoacetate has been considered as a classical example of keto-enol tautomerism. This ester is among few compounds whose keto and enol tautomers were isolated separately at low temperatures.[6] Thus, the separation of the tautomers by HPLC may be possible when column temperature is kept low. Figure 1 shows chromatograms of ethyl acetoacetate from 30 to -20 °C. A broad peak at 30 °C splits into two sharp peaks at -20 °C. The small peak corresponds to the enol tautomer because the keto form is a predominant species for this ester. The percentage of enol tautomer in Fig. 1 is 8.5% which is in good agreement with the data of other authors (7-13%[7]).

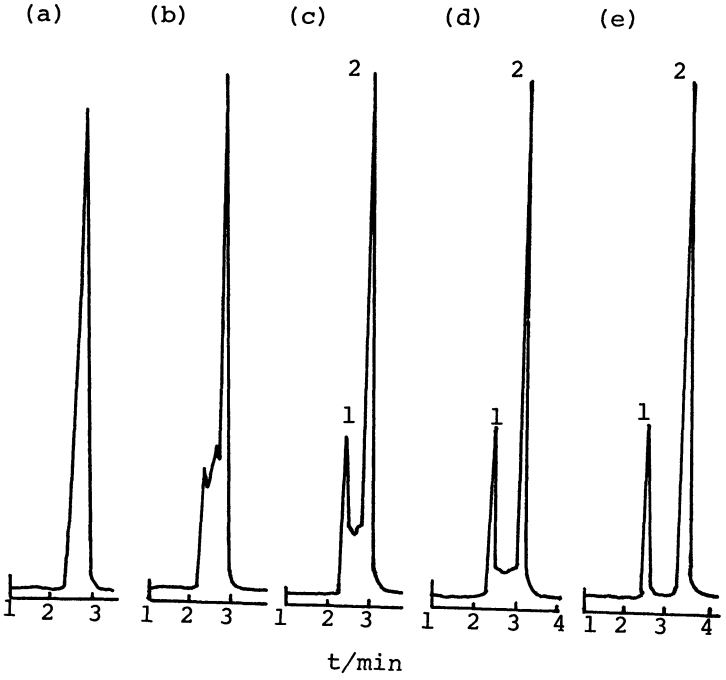


Fig. 1. Separation of keto and enol isomer of ethyl acetoacetate.

1: enol form, 2: keto form.

Column: Polygosil 60-5(4.6 mm x 15 cm),
Eluent: hexane:1-propanol:acetic acid = 100:10:3, 2.5 cm^3/min,
Detector: UV 270 nm,
Column temperature: (a) 30 °C; (b) 20 °C; (c) 10 °C; (d) 0 °C; (e) -20 °C,
Sample: pure ethyl acetoacetate, 2.5 µl.

2,4-Pentanedione(acetylacetone) has been well known as one of the most common chelating reagents in analytical chemistry. The ratio of two tautomers is sensitive to solvent composition and enol content is high in polar solvents. Thus, keto form is predominant in hexane, while in water enol form is a predominant species. Figure 2 shows chromatograms of 2,4-pentanedione under various column temperatures. The keto and enol forms were separated completely at -50 °C. In order to examine the dependence of the ratio of the keto and enol tautomers on solvents, 2,4-pentanedione was dissolved in a variety of solvents (5%, 20 °C), and these samples were supplied to HPLC at -50 °C. Figure 3 exemplifies chromatograms. The contents of the enol tautomer in hexane, carbon tetrachloride, ethanol, acetic acid, water, and in pure state were calculated to be 97.5, 96, 83, 79, 27, and 82%, respectively. These results are in good agreement with the data reported so far,[7] considering the fact that the contents are affected by the concentration as well as solvent composition.

Different from β-diketones, the content of enol tautomer is low for simple ketones. NMR is not available to determine the ratios of two isomers when the content of enol form is very low. Thus, determination of enol content of simple ketones has been tried by many workers based on bromine titration under carefully

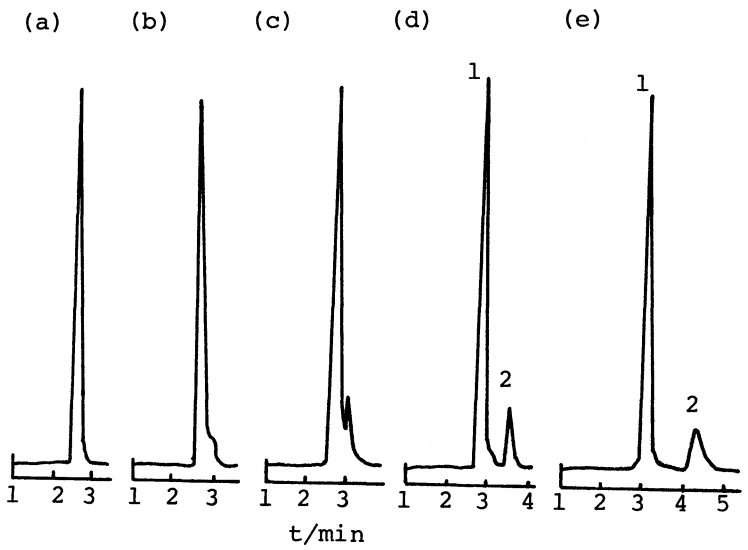


Fig. 2. Separation of keto and enol isomer of 2,4-pentanedione.

1: enol form, 2: keto form.

Column: LiChrosphere SI 1000 (4.0 mm x 25 cm),
Eluent: hexane:1-propanol:acetic acid = 100:10:3, 2.5 cm^3/min,
Detector: UV 320 nm,
Column temperature: (a) 20 °C; (b) 0 °C; (c) -10 °C; (d) -30 °C; (e) -50 °C,
Sample: pure 2,4-pentanedione, 2 µl.

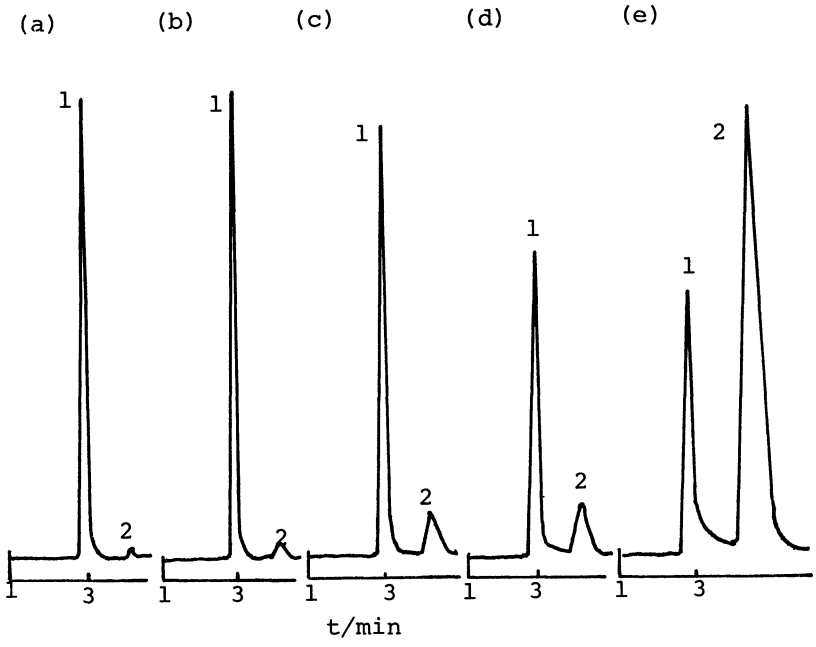


Fig. 3. HPLC chromatograms of 2,4-pentanedione in various solvents.

1: enol form, 2: keto form.
Sample: (a) in hexane; (b) in carbon tetrachloride; (c) in ethanol; (d) in acetic acid; (e) in water,
Detector: (a)-(d) 320 nm; (e) 270 nm,
Sample size: (a)-(d) 5 µl; (e) 0.2 µl,
Column temperature: -50 °C.
Other HPLC conditions, see Fig. 2.

developed conditions.[8,9] These results seem to include large error due to the difficulty to trace fast reactions based on the conventional technique. For example, the content of enol form of cyclohexanone ranges from 0.0004 to 1.2%.[7]

Pure cyclohexanone was chromatographed at various column temperatures, the results being depicted in Fig. 4. A small peak appeared on chromatograms when column temperature was below -45 °C, along with a large peak attributed to keto form of cyclohexanone. When the portion corresponding to the former small peak in Fig. 4(f) was collected and rechromatographed, the same chromatograms as in Fig. 4(f) was obtained again. Thus, the former small peak is not attributed to the impurity but to a dynamic isomer existed in the equilibrium state. Since the rate of inversion of cyclohexane ring is very fast and the possibility of other

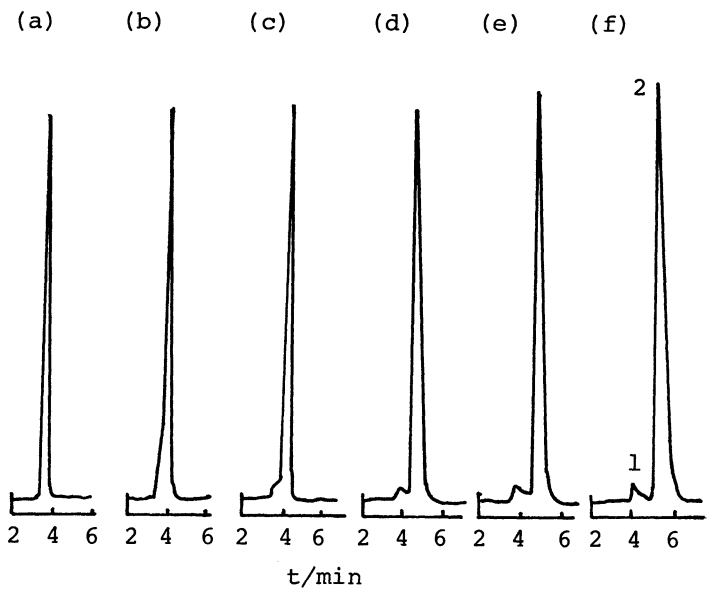


Fig. 4. Separation of keto and enol isomer of cyclohexanone.

1: enol form, 2: keto form.
Column: Polygosil 60-5(4.6 mm x 15 cm),
Eluent: hexane:1-propanol:acetic acid = 100:5:1.5, 2.0 cm^3/min,
Detector: UV 285 nm,
Column temperature: (a) -20 °C; (b) -30 °C; (c) -40 °C; (d) -50 °C; (e) -55 °C; (f) -60 °C,
Sample: pure cyclohexanone, 2.5 µl.

isomerism is unlikely, the present phenomenon will be well interpreted in terms of keto-enol tautomerism. The content of enol form in Fig. 4(f) amounts to approximately 1.5%, which is fairly in good agreement with the results shown in Ref. 10.

Cyclooctanone was found to give similar chromatogram patterns at -60 °C. Contrary to this, acetone gave only one peak at -60 °C. This will be due to the fact that enol content of acyclic simple ketones is much smaller than that of cyclic ketones.[7] We consider that HPLC is a powerful tool for investigating keto-enol tautomerism as well as NMR.

References

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