  Bull. Chem. Soc. Jpn., 58, 3351—3355 (1985) 

# Kinetic Studies of Fast Equilibrium by Means of High-performance Liquid Chromatography. XII.[1] Rate Constants and Energy Barriers of Intramolecular Carbonyl-Nitrogen Bond Rotation of *o*-Substituted Acetanilides

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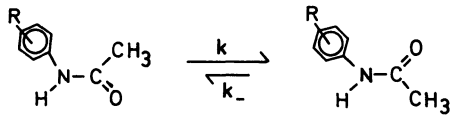
(Received April 24, 1985)

A novel high-performance liquid chromatographic(HPLC) method has been proposed to determine the rate constants and activation energies of the interconversion between two labile species existing in equilibrium in solution. The rotational energy barriers to intramolecular bond rotation about the carbonyl-nitrogen bonds of several *o*-substituted acetanilides have been determined by this method. These energy barriers have been found to increase when bulkier groups are attached at the *o*-position on the benzene ring. These have also been determined by a dynamic nuclear magnetic resonance spectroscopic(DNMR) method, which gives good agreement with the HPLC results.

In our previous reports,[2,3] dynamic equilibria between two rotamers (*E*- and *Z*-form) of several anilides including formanilide and acetanilide derivatives have been investigated by means of high-performance liquid chromatography(HPLC). HPLC




E-form Z-form

of several anilides at low temperatures have led to the successful separation of two labile rotamers. We have called the HPLC method dynamic high-performance liquid chromatography(DHPLC) in imitation of dynamic NMR. When the interconversion is relatively slow even at room temperature, either of the equilibrated species can be enriched by HPLC. In this case the rate constants and energy barriers to the interconversion can be easily determined.[4] On the other hand, when the interconversion is faster, as is the case for formanilide[2] and metal dithiocarbamates,[5] this treatment failed at room temperature, but was found possible under cooled conditions. This treatment was found to be inapplicable to acetanilides as the interconversion between the two rotamers of acetanilides are much faster than in the case of formanilide. Thus, when each of the portions collected were rechromatographed, howsoever carefully and quickly the experiments were carried out, almost equilibrated chromatograms were always obtained even at low temperatures. The difficulty has forced us to devise another method to determine the rate constants and energy barriers to the bond rotation. In this report, an approximate HPLC method is proposed to determine the energy barriers from the change in the HPLC chromatograms. We have determined the rate constants and energy barriers of intramolecular bond rotation of several *o*-substituted acetanilides. We have also determined the energy barriers by DNMR and compared both results. The attempt to determine the energy barriers in other acetanilides such as *m*- or *p*-substituted ones by means of HPLC was unsuccessful because the content of the *E*-form is low for these acetanilides.

## Experimental

2-Methylacetanilide, 2-ethylacetanilide, 2-isopropylacetanilide, and 2,4-dimethylacetanilide were prepared by usual procedures from the corresponding aromatic amines and acetic anhydride.[6] The samples were twice recrystallized from hexane–acetone. The HPLC apparatus for use in low-temperature measurements was described in our previous communications.[3,5] The column effluent was passed through a narrow stainless steel tube (0.5 mm in internal diameter and 10—20 m in length) which was thermostated at 50—100 °C in an air bath prior to detection by means of a UV detector.[3] This treatment, however, was found not always to be necessary in this case because the ratios of the two rotamers were found to be unchanged in the presence and absence of heating. This suggests that either the absorption coefficients of the two rotamers are almost the same or the re-equilibrium is attained promptly before the column effluent reaches the cell of the UV detector even in the absence of the heating. The NMR instrument (Varian XL-200) was also similar to the one described previously.[3] In order to determine the energy barriers to bond rotation, the DNMR method was applied as follows: Total line shape analysis was carried out according to the DNMR3-IT$_2$[7] program after modifying the input and output modes partially. The calculation was made on an NEC ACOS series 77 system 1000 type computer at the Computation Center of Osaka University.

## Theoretical

When the system composed of dynamic equilibria in solution is chromatographed, the separation and the interconversion will compete and complicated chromatograms may be obtained. This phenomenon

is sometimes observed in TLC,[8] GC,[9,10] and HPLC[11] included in our previous study.[2,5] In our series of measurements, we demonstrated the use of the HPLC method to investigate fast equilibria in solution. Two equilibrated species ($X \underset{k_-}{\overset{k}{\rightleftharpoons}} Y$) exist in solution, interconversion between two labile isomers being an example. The interconversion is assumed not to be rapid as compared with the separation speed by HPLC. Thus, when HPLC is carried out at low temperature, the two labile rotamers will be separated completely and interconversion between two isomers will not occur during chromatography (Fig. 1(a)). When the column temperature is slightly raised, interconversion between two labile species will occur to some extent. In this case, as shown in Fig. 1(b), the base line separation of the two species is no longer attained. When the interconversion X→Y (or Y→X) partially occurs during chromatography, the formed species Y (or X) will be eliminated from X (or Y) promptly, provided that the intrinsic retention time of X and Y differ markedly from one another. Thus, the peak area of X ($=S_X$) indicates the residual amount of X at a definite time $t$. The value $t$ is approximated from the retention time of X ($=t_r$) as follows: When a sample composed of X and Y is introduced to HPLC from an injector, it reaches the column head after the lapse of a slight interval, $t_1$, and the separation process then begins with the slight interconversion between the two rotamers being attained during chromatography. After the lapse of time, $t$, X reaches the column end and the separation stops. It will still take some more time, $t_2$, before X reaches the cell of the detector. Assuming that the peak shape is symmetrical and that the separation is attained promptly near the column head, the following equation will apply: $t_r=t_1+t_2+t$. The residual amount of X at a time $t$ is proportional to the product $S_X$ in Fig. 1(b) and the flow rate $v$. When the flow rate is decreased with the column temperature being kept constant, a larger part of X should transform into Y during the chromatographic process. Thus, from the plot $S_X v$ *vs.* $t$, the rate constant of the bond rotation can be calculated. It is noteworthy here that in this method the transformed species is promptly eliminated from the peak and thus the effect of the reverse reaction can be approximately neglected, which differs from other kinetic methods. When the rate constants are measured at a variety of column temperatures, the rotational energy barrier can be calculated from the Arrhenius' plot.

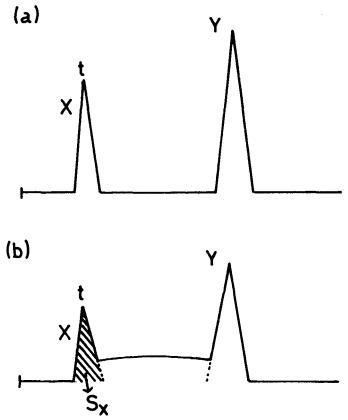



Fig. 1. Possible chromatogram patterns with partial interconversion during chromatography.

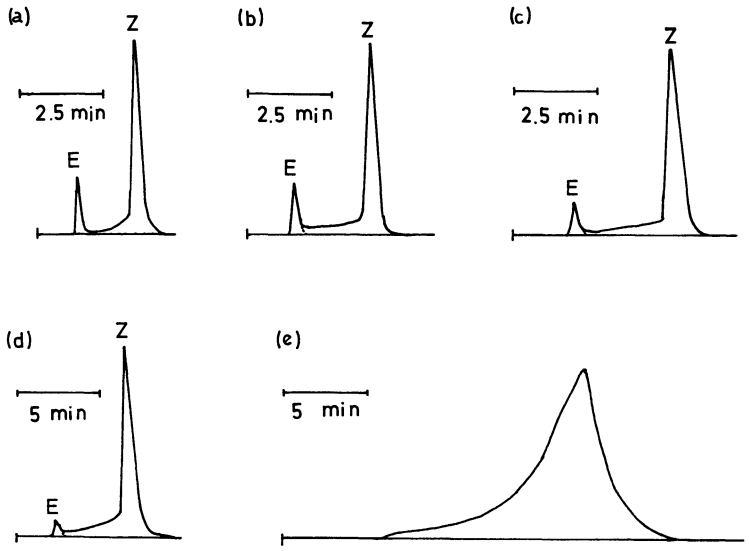



Fig. 2. HPLC chromatograms of 2-methylacetanilide at −35 °C under different flow rates. Flow rate: (a): 3.2 $cm^3\ min^{-1}$, (b): 2.5 $cm^3\ min^{-1}$, (c): 1.9 $cm^3\ min^{-1}$, (d): 1.1 $cm^3\ min^{-1}$, (e): 0.4 $cm^3\ min^{-1}$. For other HPLC conditions, see text.

## Results and Discussion

*HPLC of 2-Methylacetanilide.* As shown in the theoretical section, it is necessary to determine the void volume from the injector to the column head and from the column end to the cell of the UV detector. This was determined to be 0.25 $cm^3$ for the present HPLC apparatus by connecting a voidless 2-way union instead of the column and then injecting a sample to be detected. Thus, $t_1+t_2$ was calculated by dividing the dead volume by each flow rate.

Then, 2-methylacetanilide was chromatographed at low temperatures. The two rotamers of 2-methylacetanilide were separated under the HPLC conditions described previously;[3] solvent system: hexane:1-propanol:acetic acid=100:10:3; column: Polygosil 60—5 (4.6 mm×15 cm); flow rate: 2.0 $cm^3$ $min^{-1}$; detection: UV 254 nm. Under these conditions and the column temperature at −50 °C, base line separation of the two rotamers was achieved, and the equilibrium constants prior to HPLC could be determined. On the other hand, when the column temperature was higher than −20 °C, the two peaks coalesced, and consequently only one broad peak appeared on the chromatograms. Between these two extremes, two peaks, the larger one corresponding to the *E*-rotamer and the smaller one to *Z*-rotamer, appeared on the chromatograms with slight interconversion being attained during chromatography.[3] Then, the column temperature was adjusted from −40 to −25 °C, at various flow rates (0.3—4.0 $cm^3$ $min^{-1}$). The change of chromatograms at −35 °C is depicted in Fig. 2. With increasing flow rate, the interconversion is suppressed. The product of the peak area and the flow rate $S_X v$ is plotted against the residence time $t$ and the results is shown in Fig. 3. From the slope of these linear lines, the rate constant $k$ corresponding to the conversion from *E*-to *Z*-form at each temperature was determined. The results were $5.2\times10^{-3}$, $1.0\times10^{-2}$, $2.0\times10^{-2}$, and $3.7\times10^{-2}$ $s^{-1}$ at −40, −35, −30, and −25 °C, respectively. In Fig. 3, it is noteworthy that when the same amount of the sample was injected the extraporated values of $S_X v$ at $t=0$ were similar at different temperatures. This can reasonably be attributed to the fact that the initial content of the *E*-form before chromatography is similar for each sample.

With similar procedures, the reverse rate constant $k$ from *Z*- to *E*-form at each temperature was deter-

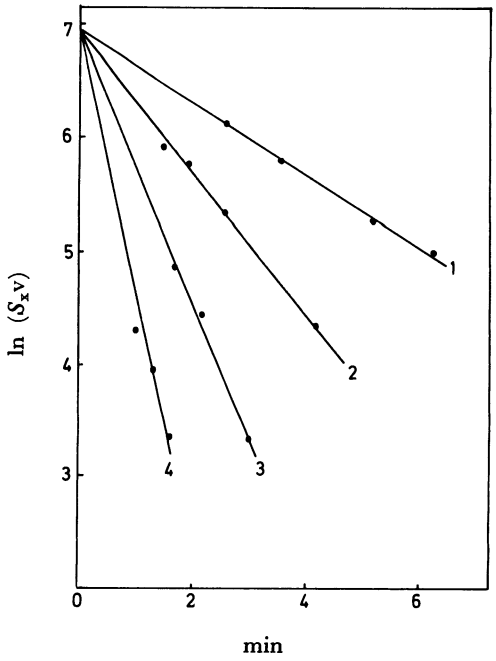



Fig. 3. Plots of residual amount of *E*-form of 2-methylacetanilide *vs.* residence time.
1: −40 °C, 2: −35 °C, 3: −30 °C, 4: −25 °C.

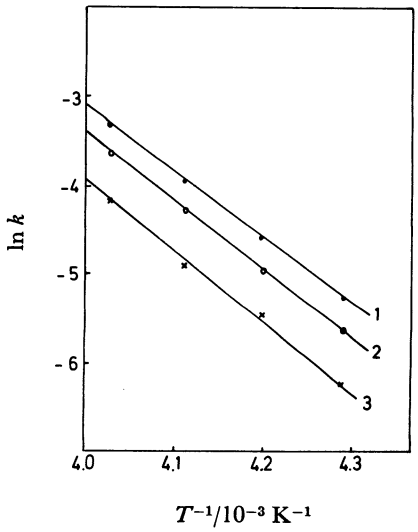



Fig. 4. Arrhenius' plot of *o*-substituted acetanilides by HPLC.
1: 2-Methylacetanilide, 2: 2-Ethylacetanilide, 3: 2-Isopropylacetanilide.

Table 1. Rate constants $k$ and $k_-$ of *o*-substituted acetanilides

| | $k(s^{-1})$ | $k_-(s^{-1})$ | $K_{calcd}=k/k_-$ | $K_{obsd}$ | | |
|---|---|---|---|---|---|---|
| | | | | −50 °C | 0° C | 25 °C |
| 2-Methylacetanilide | $5.2\times10^{-3}$ | $6.1\times10^{-4}$ | 8.4 | 4.5 | 4.4 | 4.4 |
| 2-Ethylacetanilide | $2.6\times10^{-3}$ | $3.8\times10^{-4}$ | 6.8 | 3.4 | 3.4 | 3.2 |
| 2-Isopropylacetanilide | $2.0\times10^{-3}$ | $2.5\times10^{-4}$ | 7.8 | 2.6 | 2.5 | 2.6 |

Table 2. Energy barriers of bond rotation of *o*-substituted acetanilides

| | $E_a$ (kJmol$^{-1}$) | | | |
|---|---|---|---|---|
| | HPLC[a] (−20——40 °C) | | NMR[b] (10—50 °C) | |
| | $E \rightarrow Z$ | $Z \rightarrow E$ | $E \rightarrow Z$ | $Z \rightarrow E$ |
| 2-Methylacetanilide | 62.4 | 59.0 | 74.5 | 76.4 |
| 2-Ethylacetanilide | 65.7 | 65.8 | 76.9 | 78.8 |
| 2-Isopropylacetanilide | 68.2 | 66.5 | 84.0 | 82.0 |
| 2,4-Dimethylacetanilide | 61.6 | — | — | — |

a) In eluent (hexane : 1-propanol : acetic acid = 100 : 10 : 3). b) In $CDCl_3$.

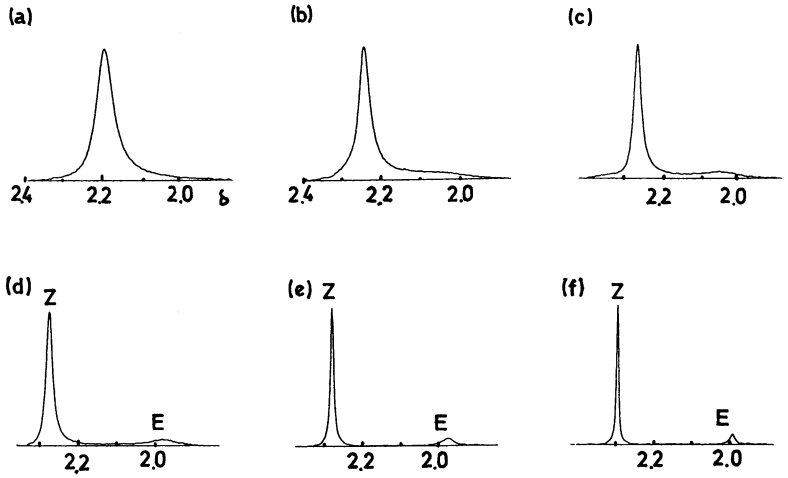



Fig. 5. $^1$H-NMR spectra of 2-ethylacetanilide in $CDCl_3$ at different temperatures.
(a): 50 °C, $k = 6.7 \times 10^1$ s$^{-1}$, (b): 40 °C, $k = 2.7 \times 10^1$ s$^{-1}$, (c): 35 °C, $k = 1.6 \times 10^1$ s$^{-1}$, (d): 30 °C, $k = 9.8 \times 10^0$ s$^{-1}$, (e): 20 °C, $k = 3.4 \times 10^0$ s$^{-1}$, (f): 10 °C.

mined to be $6.1 \times 10^{-4}$, $1.0 \times 10^{-3}$, $1.6 \times 10^{-3}$, and $3.2 \times 10^{-3}$ s$^{-1}$ at −40, −35, −30, and −25 °C, respectively. Thus, the equilibrium constant $K$ (=[*Z*-form]/[*E*-form]) should be from 8 to 12 over this temperature range. The equilibrium constant $K$ can be directly determined by means of HPLC by dissolving 2-methylacetanilide in an HPLC eluent and then letting it stand for enough time to attain equilibrium between the two rotamers, followed by HPLC analysis at −50 °C.[3] The equilibrium constant at 25, 0, and −50 °C was thus determined and was found to be almost the same($K$=4.5). Both results agree relatively well.

*HPLC of Other o-Substituted Acetanilides.* Using the procedure described above, the rate constants for the bond rotation were determined for other *o*-substituted acetanilides at low temperatures(−40 to −25 °C). The rate constants $k$ and $k_-$ at −40 °C are summarized in Table 1. These results suggest that when bulkier groups are attached at the *o*-position of the phenyl ring, intramolecular bond rotation becomes slow.

*Energy Barriers to the Bond Rotation.* Figure 4 indicates Arrhenius' plots ($k$ *vs.* $1/T$) for *o*-substituted acetanilides. From the slope of these linear lines, the energy barriers to the bond rotation from the *E*- to the *Z*-form were calculated; they were found to increase slightly when bulkier groups were substituted. Using similar procedures, the energy

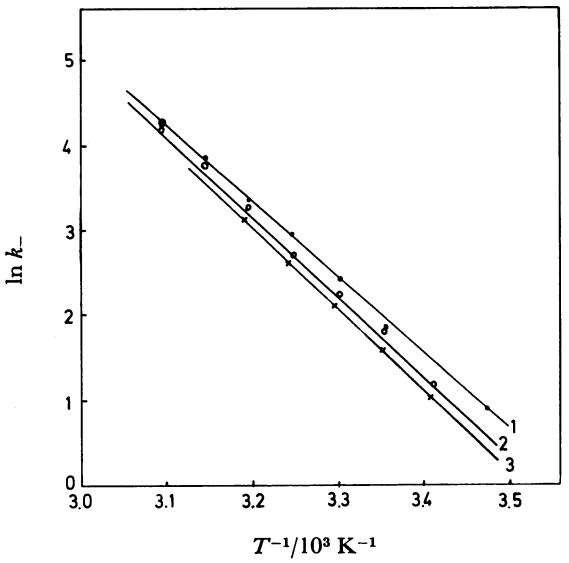



Fig. 6. Arrhenius' plot of *o*-substituted acetanilides by $^1$H-NMR.
1: 2-Methylacetanilide, 2: 2-Ethylacetanilide, 3: 2-Isopropylacetanilide.

barriers of the reverse reaction (from *Z*- to *E*-form) were determined. These results are summarized in Table 2. The energy barriers of the bond rotation of forward and backward reactions were found to be closely similar.

*DNMR Measurements.* Previous NMR study have also demonstrated the presence of both *E*- and *Z*-forms for *o*-substituted acetanilides[12] but evaluation of the energy barriers by the DNMR method was not carried out as the percentages of the *E*-form are low, and consequently, total line shape analysis was difficult using relatively low field NMR instruments with low sensitivity. Figure 5 indicates 200 MHz $^1$H-NMR spectra of 2-ethylacetanilide for the acetyl protons from 10 to 50 °C. Two sharp signals ($\delta$=2.28 and 1.98 for *Z*- and *E*-form, respectively) at 10 °C are broadened with the rise of temperature and at 50 °C the two peaks coalesce. It is interesting to note that the change in the NMR spectra (Fig. 5) resembles that of the HPLC chromatograms (Fig. 2). The rate constants for the bond rotation at each temperature were determined by total line shape analysis (Fig. 5). The energy barrier corresponding to the conversion from the *Z*- to the *E*-form was calculated to be 78.8 kJ mol$^{-1}$ from the Arrhenius' plots (Fig. 6). Similar experiments were carried out for 2-methylacetanilide and 2-isopropylacetanilide. The rate constants $k_-$ at −30 °C were determined to be $1.1\times10^1$, $9.8\times10^0$, and $9.4\times10^0$ s$^{-1}$, for 2-methylacetanilide, 2-ethylacetanilide, and 2-isopropylacetanilide, respectively. The energy barriers were also calculated and the results are shown in Table. 1. In both NMR and HPLC the energy barriers are found to increase when bulkier substituents are introduced at the *o*-position. The energy barriers obtained by HPLC are somewhat smaller than those by NMR, which may be interpreted at least partially in terms of the difference in the polarity of the solvents and the temperature range. If these conditions are taken into consideration, both results agree fairly well. The energy barriers obtained by the present HPLC method are not always accurate because it may sometimes occur that the bond rotation is catalytically accelerated or suppressed on the surface of the adsorbent silica-gel. The authors, however, consider that the HPLC method will be a useful method for approximately estimating the energy barriers of fast equilibria.

The authors wish to express their gratitude to Dr. Usha Shome, National Botanical Research Institute, Lucknow, India, who kindly corrected the English of this paper.

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