Kinetic Studies of Fast Equilibrium by Means of High-performance Liquid Chromatography. VI. Separation of Rotamers of Formanilide

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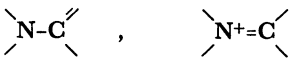
The restricted rotation about the carbon-nitrogen bond of formanilide has been investigated by high-performance liquid chromatography. At lower temperatures, two peaks corresponding to two dynamic rotamers (*E*- and *Z*-form) have appeared on chromatograms, whereas at higher temperatures they have not been separated and a single peak has appeared on chromatograms, due to rapid bond rotation. The equilibrium concentration ratio of *E*- to *Z*-form has been found to vary in different solvents, suggesting the presence of solute-solvent and solute-solute interactions such as hydrogen bond formation. Determination of kinetic characteristics such as rate constant and activation energy of fast reactions has been established by high-performance liquid chromatography. The authors have named the present method "dynamic high-performance liquid chromatography," in imitation of "dynamic nuclear magnetic resonance spectroscopy" which traces fast equilibrium.

In this research series[1-5] a method has been devised to investigate fast equilibrium by means of high-performance liquid chromatography(HPLC). In part IV of this series,[4] the restricted rotation about carbon-nitrogen bonds of unsymmetrical palladium(II) dithiocarbamate chelates was examined. The carbon-nitrogen bonds of these compounds have the double-bond character shown in the following cannonical forms, and these compounds gave two peaks in



HPLC chromatograms at lower temperatures due to the separation of two labile rotamers. On the other hand, at higher temperatures rotamers were not separated and only one peak appeared.

There exist numerous substances which have a partially double-bond character on carbon-nitrogen bonds. Amides fall under this category. The present report deals with a kinetic study of intramolecular bond rotation of formanilide.

Experimental

Reagents. Formanilide was purchased commercially and was recrystallized from hexane-diethyl ether. Acetanilide was also obtained commercially and was used without further purification.

Apparatus. The HPLC apparatus described in our previous report[4] was modified as depicted in Figs. 1 and 2. Bathcooler (Model Cryocool CC-80f, Neslab Instrument Inc.), thermoregulator, heater, stirrer and HPLC column were plunged into the bath filled with methanol. The temperature of the bath was controlled from 25 to −60 °C. HPLC was possible up to −60 °C with a gradual decrease of column efficiency. Satisfactory separation of two rotamers was attained when temperature was below −30 °C. Two injectors (both Model 7125, Rheodyne), a six-way valve (Model 7010, Rheodyne), and a three-way valve (Model 7010, Rheodyne) were fixed on the plastic board which was placed on the upper part of the bath. Thus, most of the flow paths in stainless steel tubes were immersed in the cooled bath.

In order to obtain kinetically unstable species and to observe its change into other species, the following procedures were carried out. The equilibrated solution of two isomers (X⇌Y) was supplied to HPLC from injector I, and the portion corresponding to the retention time of X (or Y) was collected into the reservoir, which was controlled at various temperatures(−30—0 °C) by a freezing mixture composed of methanol-water. The collected portion was stirred and then kept standing for a few minutes. After the flow paths were changed by the six-way valve, the solution in the reservoir was sucked in from injector II. The injection was repeated for a definite interval. With the lapse of time, the peak of Y (or X) grew, while that of X (or Y) fell. Thus, the progress of the reaction X→Y (or Y→X) at lower temperatures was traced by HPLC.

Nuclear magnetic resonance (NMR) spectra were measured on a Varian Model XL-200 instrument, using tetramethylsilane as an internal reference.

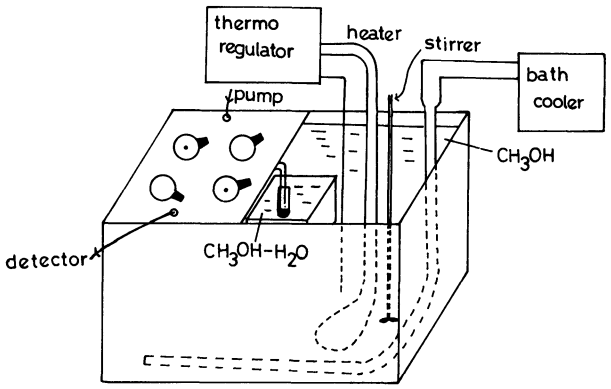


Fig. 1. HPLC apparatus for low temperature measurements.

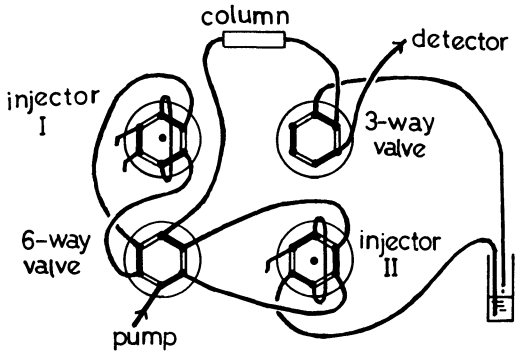
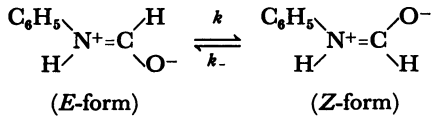


Fig. 2. Flow path of HPLC sampling system.

Results and Discussion

HPLC of Formanilide at Lower Temperatures. The theoretical treatment for analyzing a fast unimolecular reaction by HPLC was detailed in our previous report.[4]

Figure 3 shows chromatograms of the mixture of formanilide and acetanilide on silica gel packings at various temperatures. A slight broad peak of formanilide at room temperature splits into two sharp peaks at −30 °C *via* complicated chromatograms at intermediate temperatures. Contrary to this, acetanilide gives only one peak at all temperatures. This phenomenon is explained as follows. It has been established from NMR study that amide compouds can exist as the equilibrated mixture of two dynamic rotamers.[6] Thus, the NMR study[7] has demonstrated that formanilide exists as the mixture of two rotamers (*E*- and *Z*-form), while acetanilide exists exclusively as the *Z*-form.



(1)

Since the carbon-nitrogen bond has a strong double-bond character, the intramolecular bond rotation around this carbon–nitrogen bond is seriously restricted. Figure 3 suggests that this bond rotation does not occur during chromatography at lower temperatures and then two rotamers are separated. On the other hand, at higher temperatures, since partial or complete bond rotation occurs during chromatography, two rotamers are not separated.

Equilibrium Ratio of Two Rotamers. Since the bond rotation practically does not occur during chromatography when HPLC is operated under −30 °C, it follows that chromatograms obtained at this temperature directly indicate the equilibrium concentrations of two isomers prior to HPLC. Thus, when samples of formanilide dissolved in different conditions, such as concentration, solvent and temperature, are submitted to HPLC, chromatogram patterns will vary due to the differences of the equilibrium ratios of the two isomers.

Formanilide was dissolved in 22 different solvents and these solutions were submitted to HPLC at −30 °C. Figure 4 exemplifies chromatograms of 0.5% formanilide solutions in different solvents. Here the temperatures of the sample solutions were controlled to be 25 °C. The ratio of two peak heights was found to vary markedly. Now, since it was elucidated that the equilibrium ratio of two isomers really varied in different solvents, an attempt was made to determine the equilibrium ratio of two isomers in different conditions; the procedure was carried out as follows, similar to that described in our previous report.[4] The equilibrated sample solution was submitted to HPLC and the portions corresponding to the former and the latter peaks were collected. The solution was then diluted to a definite volume by HPLC solvent. Each solution reached equilibrium promptly at room temperature according to Eq. 1. Thus, when the aliquot of these solutions was submitted to HPLC once more, two equilibrated peaks appeared on chromatograms again. Thus, from the measurements of two peak heights (or areas), the ratio of two isomers was determined. It should be noted here that direct measurements of two peak areas in Fig. 4 do not give the ratio of two isomers due to the difference in their absorption coefficients.

In order to identify the peaks which appeared on chromatograms, we compared present HPLC results with NMR results reported by other authors.[7] It was demonstrated in Ref. 7 that in chloroform the ratio of *Z*-form of formanilide increased with increase of form-

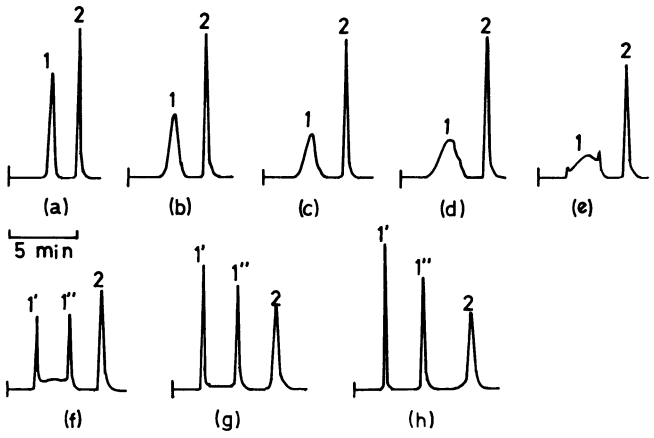


Fig. 3. HPLC chromatograms of the mixture of formanilide and acetanilide at various temperatures. Column: Polygosil 60–5 (4.6 mm × 25 cm). Eluent: hexane: isopropyl acetate: acetic acid = 100: 47: 3. Flow rate: 2.5 cm³/min. Detector: UV 254 nm. (a) 25°C, (b) 15°C, (c) 10°C, (d) 5°C, (e) 0°C, (f) −10°C, (g) −20°C, (h) −30°C. 1 formanilide (1′ *E*-form, 1″ *Z*-form), 2 acetoanilide.

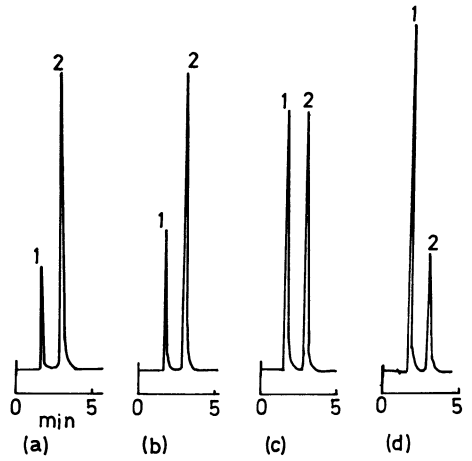


Fig. 4. HPLC chromatograms of 0.5% solution of formanilide dissolved in different solvents. Colum: Polygosil 60–5 (4.6 mm × 25 cm). Eluent: hexane: ethyl acetate: acetic acid = 100: 57: 3. Flow rate: 2.5 cm³/min. Detector: UV 254 nm. Column temperature: −30 °C. (a) in 2-propanol, (b) in methanol, (c) in acetic acid, (d) in carbon tetrachloride. 1 *E*-form of formanilide, 2 *Z*-from of formanilide.

anilide concentration. Formanilide was dissolved in chloroform at various concentrations to be submitted to HPLC. The ratio of the two peak areas was found to vary according to the concentration of formanilide, and the ratio of the latter peak was large when concentration of formanilide was high. The latter peak was thus identified as *Z*-form by NMR study, as shown in Table 1.

Now that the method to determine the ratio of two isomers was established and the problem of peak identification was resolved, equilibrium ratios of two isomers in 22 different solvents were determined; the results are summarized in Table 2. Interesting is the fact that when formanilide is dissolved in polar solvents such as methanol and water, the percentage of *Z*-form is high, while in nonpolar solvents such as chloroform and carbon tetrachloride, *E*-form is the predominant species. The latter phenomenon will be interpreted in terms of solute–solute hydrogen bond formation; *E*-form of formanilide exists as a ring dimer in chloroform or carbon tetrachloride.[7-10] This effect seems to result in a more stable structure of *E*-form in these solvents. In the former, polar solvents such as methanol and water will interact with formanilide strongly by solute–solvent hydrogen bond formation. When *Z*-form of formanilide enters into the tetrahedral lattice in solvent water, this species will be stabilized by hydrogen bond formation, as shown in the following

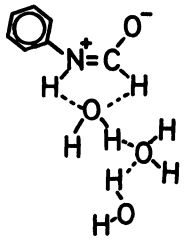


chart. A similar discussion will hold for several other polar solvents, such as methanol and ethanol.

Rate of Interconversion and Energy Barrier for Bond Rotation. The rate constant k in Eq. 1 was determined by HPLC with similar procedures as described in our previous report.[4] The equilibrated solution of formanilide was supplied to HPLC through injector I, and the portion corresponding to the former peak (*E*-form) was collected into a reservoir thermostated at low temperatures. The collected portion was stirred and then left standing for a few minutes. After changing the flow path by the six-way valve, the solution in the reservoir was sucked into the column from injector II repeatedly at a definite intervals. With the lapse of time, the peak of *Z*-form appeared and grew gradually, while the peak of *E*-form fell. The rate constant k was calculated according to the following equation assuming a unimolecular process:[4]

$$k = \{y_e/x_0(t'+\Delta t)\}\ln\{y_e/(y_e-y'-\Delta y)\}, \quad (2)$$

where x_o, y_e and $y'+\Delta y$ were the initial concentration of *E*-form, the equilibrium concentration of *Z*-form and the concentration of *Z*-form at a definite time, $t'+\Delta t$, respectively. Thus, from the growth of peak height of *Z*-form, k was calculated. The value at −27.6 °C was determined to be $(1.27\pm0.05)\times10^{-3}$ s^{-1}.

In order to determine the energy barrier(E_a) for the

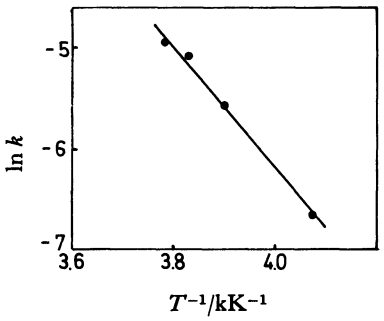


Fig. 5. Activation energy of the carbon–nitrogen bond rotation. Chromatographic conditions were similar to those shown in Fig. 4. The values of k were determined at various temperatuers according to Eq. 3. The value E_a was determined from the follwoing equation: $k=A_0\exp(-E_a/RT)$

Table 1. Percentage of formaniliide existing as *Z*-form *vs.* concentration in chloroform

mol%	HPLC(25 °C)* *Z*-form %	mol%	NMR(35 °C)** *Z*-form %
		31.3	64
28.7	63		
		23.3	62
16.7	60		
		9.7	58
9.1	54		
		5.1	50
4.8	47		
		2.9	48
2.5	46		
		1.5	45

* This work. ** Ref. 7. These data are values in deuterated chloroform.

Table 2. Percentage of formanilide existing as *Z*-form in different solvents

Solvent	*Z*-form %	Solvent	*Z*-form %
2-propanol	81	dimethyl sulfoxide	65
1-butanol	79	benzene	58
ethanol	78	hexane*	57
N,*N*-dimethyl formamide	74	formamide	57
water	73	1,4-dioxane	57
pyridine	71	cyclohexane*	54
methanol	71	dichloromethane	53
acetonitrile	70	acetic acid	49
tetrahydrofuran	70	acetone	48
ethyl acetate	68	chloroform	46
diethyl ether	66	carbon tetrachloride	28

0.5% solution, 25 °C. * 0.1% solution.

carbon–nitrogen bond rotation, k was measured at different temperatures. Thus, from Arrhenius' plot shown in Fig. 5, E_a was determined to be (54.2±5.0) kJ mol^{-1}. From this plot the rate constant k at room temperature was estimated to be 9.5×10^{-2} s^{-1} (at 25 °C). Thus, it follows that the bond rotation occurs several times in a minute at room temperature.

Comparison of HPLC and NMR Results. The ^{1}H NMR spectra of formanilide were measured in deuterated dimethyl sulfoxide at various temperatures. A part of the spectra concerning the C–H protons near 8.3 and 8.8 ppm, assigned as *Z*- and *E*-form, respectively, is depicted in Fig. 6. The lower field band of *E*-form gave triplet-like signals at room temperature, which were analyzed as a mixture of doublet and singlet signals; the former was coupled with N–H proton (J=11.2 Hz) and the latter was not because of being replaced by deuteron in the solvent. When the temperature was increased to 60 °C, the "triplet" signals coalesced into a broad signal. A similar discussion will hold for the *Z*-form signal of C–H (8.3 ppm), though due to small spin–spin coupling constant, this band gave a singlet peak with deuteron exchanged partially. The spectral pattern from 60 to 150 °C shown in Fig. 6 resembles that of HPLC chromatograms depicted in Fig. 3, though the temperature range differs. This can reasonably be interpreted as follows. NMR has been well known to be a powerful tool for investigating fast equilibrium in solution, because two conformers which undergo interconversion promptly can be detected as different two species. Thus, two isomers of formanilide can be distinguished by NMR at room temperature. With increasing temperature, since the rate of interconversion increases rapidly, the two signals will commence to coalesce. A similar phenomenon should also occur in HPLC. Since a kinetically unstable species whose lifetime is a few seconds cannot be distinguished by HPLC, only one peak appears on chromatograms at room temperature. With decreasing temperature, lifetimes of two rotamers are prolonged and consequently two peaks corresponding to each rotamer appear on chromatograms. Thus, both Figs. 3 and 6 reflect identical phenomenon, *i.e.*, carbon–nitrogen bond rotation.

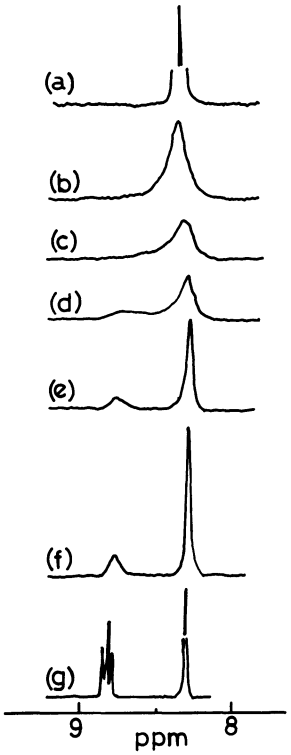


Fig. 6. The ^{1}H NMR spectra of formanilide in dimethylsulfoxide at different temperatures.
(a) 150 °C, (b) 100 °C, (c) 90 °C, (d) 80 °C, (e) 70 °C, (f) 60 °C, (g) 24 °C

The method has been well established for examining fast equilibrium of two dynamic isomers by means of NMR, which has been called dynamic nuclear magnetic resonance spectroscopy (DNMR). The energy barrier of carbon–nitrogen bond rotation has been well examined on various amides by DNMR. This method is based on the measurements of signal width at different temperatures, which is broadened due to fast interconversion of two conformers. Thus, from the signal shape analysis, the energy barrier has been determined to be from 13 to 30 kcal mol^{-1} (1 kcal mol^{-1}=4.18 kJ mol^{-1}) for various amides, mainly for *N,N*-disubstituted amides.[6] Determination of the energy barrier for *N*-monosubstitutued amides by ^{1}H NMR is difficult because intermolecular hydrogen bond formation complicates signal shape. Two isomers of *o*-methylformanilide were separated with careful treatment[11] and their ^{1}H NMR spectra were measured at low temperatures;[12] the E_a and k for this amide were 15.7 kcal mol^{-1} and $(2.7—4.4)\times10^{-3}$ s^{-1}, respectively, at −40 °C. Since two isomers of nonsubstitutued formanilide would rotate rapidly, while the *o*-methyl-substitutued one would not due to steric hindrance, the present value of E_a (54.2 kJ mol^{-1}=13.0 kcal mol^{-1}) of the former seems to be a reasonable one. DNMR study of ^{13}C NMR of formanilide demonstrated[13] that E_a was about 20 kcal mol^{-1} at high temperatures under the assumption that the effect of hydrogen-bond formation on signal shape was negligible in ^{13}C NMR study. The discrepancy between the ^{13}C NMR and present HPLC results might be interpreted at least partially in terms of the temperature difference of the measurements.

Conclusions

In this rsearch series, it has been demonstrated that rather fast equilibria can be traced by means of HPLC based on conventional principles. For this purpose, DNMR method has been used exclusively. The present HPLC method is comparable with the DNMR method. Since the time scale of detection for HPLC differs from that for NMR, kinetic data obtainable by DNMR method are not always obtained by HPLC method. The authors, however, consider that the HPLC method have some advantages over the DNMR method. First, undesirable effects such as hydrogen-bond formation, which tends to broaden the signal shape in NMR, can be neglected in HPLC. Second, in HPLC method, kinetically unstable species can be obtained, so determination of physicochemical properties of labile species will be possible by combining HPLC

with other suitable apparatus. The authors propose here to call this HPLC method dynamic high-performance liquid chromatography, in imitation of DNMR which traces fast equilibria in solution.

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