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Kinetic Studies of Fast Equilibrium by Means of High-performance Liquid Chromatography. X. Separation of Rotamers of Acetanilides

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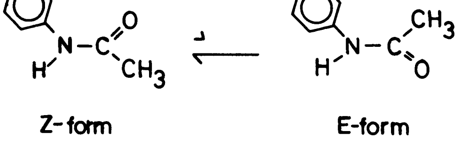
The restricted rotation about the carbonyl–nitrogen bonds of acetanilides has been investigated by means of high-performance liquid chromatography. The separation of two rotamers (*E*- and *Z*-forms) of acetanilides has been achieved at low temperatures. The presence of a very small amount of the *E*-form has been confirmed for 3-, 4-, and non-substituted acetanilides. The ratios of the *E*- and *Z*-forms in different solvents have also been determined. By combining the HPLC and NMR results, it has been elucidated that the molecular structure of acetanilide in solution can be described in terms of an equilibrium between a large amount of the nearly planar *Z*-form and a small amount of the twisted *E*-form.

In a series of papers,[1–5)] we have previously demonstrated that considerably fast equilibria in solutions can be examined by means of high-performance liquid chromatography (HPLC) based on conventional principles. We have entitled the HPLC method "dynamic HPLC"[3)] in imitation of "dynamic NMR." We have already separated two rotamers of formanilide at low temperatures and shown that the ratios of rotamers are affected by the solvent composition as well as by the concentration. The present report deals with the separation of various acetanilide derivatives.

Several reports have appeared[6–8)] on the NMR study of acetanilides in order to elucidate the conformation, and these results may be summarized as follows. Acetanilide exists as a nearly planar structure, and π-electrons of the benzene ring may be partially delocalized onto the $>\mathrm{N}^+{=}\mathrm{C}<^{\mathrm{O}^-}$ moiety, consequently forming an extended partially-conjugated system. The steric hindrance between the benzene ring and the methyl group is so large that acetanilide exists exclusively as the *Z*-form; the presence of the *E*-form has not hitherto been confirmed by NMR. The replacement of *m*-(3-) or *p*-(4-) hydrogen in the benzene ring by a bulky substituent does not bring about any remarkable change in the molecular structure. Contrary to this, when an *o*-(2-) hydrogen is substituted, a drastic change in the molecular structure results. The steric hindrance between the *o*-substituted benzene ring and the carbonyl group is so large that the planar structure is no longer maintained even in the *E*-form rotamer, and eventually the benzene ring deviates from the amide plane and a twisted structure results.[8)]



Experimental

Reagents. The following acetanilide derivatives were obtained from the market; acetanilide, 2-hydroxyacetanilide, 3-hydroxyacetanilide, 4-hydroxyacetanilide (acetaminophene), 4-ethoxyacetanilide (phenacetin), 2-nitroacetanilide, 3-nitroacetanilide, 4-nitroacetanilide, and *N*-methylacetanilide. The other acetanilides were synthesized by usual procedures from the corresponding aromatic amines and acetic anhydride;[9)] 2-methylacetanilide, 3-methylacetanilide, 4-methylacetanilide, 2-ethylacetanilide, 2-isopropylacetanilide, 2,4-dimethylacetanilide, 2,6-dimethylacetanilide, and 2,4,6-trimethylacetanilide.

Apparatus. The HPLC apparatus used in this study is similar to the one described previously.[3)] In order to determine the precise ratios of the two rotamers, some modification of the apparatus was made as follows: A column effluent was passed through a narrow stainless-steel tube (0.5 mm in diameter and 10—20 m in length) which was thermostated at 50—100 °C in an air bath (Model ASB-200, Japan Spectroscopic Co., Ltd.) prior to detection by means of a UV detector. This treatment promptly facilitated the attainment of an equilibrium between the two rotamers in the effluent. When the equilibrium is attained in the effluent, the ratio of the two peak areas should reflect the population ratio of the rotamers, irrespective of their absorption coefficients.

The proton NMR spectra of several acetanilides were measured on a Varian XL-200 spectrometer (200.06 MHz) in $\mathrm{CDCl_3}$ from 25 to −10 °C.

Results

HPLC of o-Substituted Acetanilides. Various acetanilides were dissolved in chloroform (0.5%), and these sample solutions were submitted to HPLC at different column temperatures. Figure 1 exemplifies the chromatograms of 2-methylacetanilide. A sharp

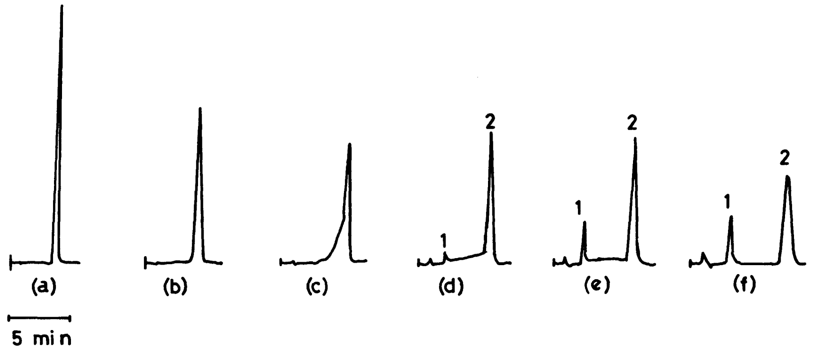


Fig. 1. HPLC chromatograms of 2-methylacetanilide at various temperatures.
Column: Polygosil 60–5 (4.6 mm × 15 cm). Eluent: hexane : 1-propanol : acetic acid = 100 : 10 : 3. Flow rate: 2.0 cm^3/min. Detector: UV 254 nm. (a): 20 °C, (b): 0 °C, (c): −20 °C, (d): −30 °C, (e): −40 °C, (f): −50 °C. 1: *E*-form, 2: *Z*-form.

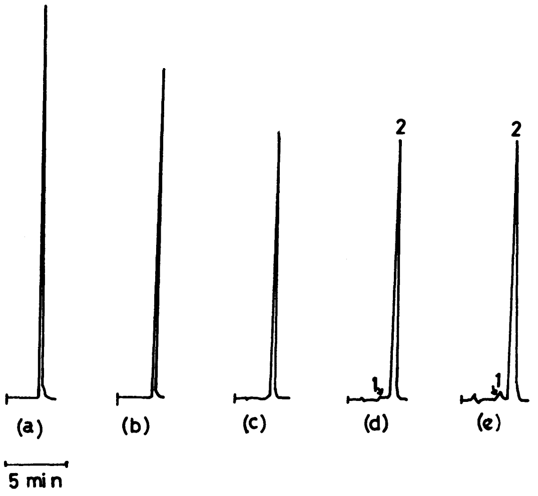


Fig. 2. HPLC chromatograms of 3-methylacetanilide at various temperatures.
For chromatographic conditions, see Fig. 1.
(a): 20 °C, (b): −10 °C, (c): −30 °C, (d): −40 °C, (e): −50 °C. 1: *E*-form, 2: *Z*-form.

peak at room temperature splits into two peaks at low temperatures. The complete baseline separation of the two peaks at −50 °C suggests that interconversion between two rotamers does not take place during chromatography at this temperature. The former and the latter peak in Fig. 1 are attributed to the *E*- and *Z*-forms respectively by comparing the present HPLC results with the NMR data[8] reported by Kessler and Rieker. Other *o*-substituted acetanilides, such as 2-ethylacetanilide and 2-isopropylacetanilide, gave similar chromatographic patterns.

HPLC of Acetanilide, 3-Methylacetanilide, and 4-Methylacetanilide. Figure 2 shows chromatograms of 3-methylacetanilide at various column temperatures. Two rotamers of 3-methylacetanilide were separated by HPLC below −50 °C. The small peak in Fig. 2(e) is not due to impurity but is attributed to the *E*-form for the following reason: When the fraction corresponding to the small peak in Fig. 2(e) was collected and rechromatographed after having been kept at room temperature for some time, equilibrated chromatograms were obtained again. Quite similar chromatograms were obtained when 4-methylacetanilide and acetanilide were chromatographed. Thus, the presence of a very small amount of the *E*-form isomer was confirmed for these acetanilides.

HPLC of Other Acetanilides. When two or more hydrogens of the benzene ring are replaced by bulky substituents, the chromatographic patterns might vary. 2,4-Dimethylacetanilide gave chromatograms quite similar to 2-methylacetanilide. Thus, the equilibrium between *E*- and *Z*-rotamers is determined by the *o*-methyl group, and the *p*-methyl group seems to have little effect on the ratio. 2,6-Dimethylacetanilide and 2,4,6-trimethylacetanilide gave chromatograms similar to 2-methylacetanilide, though the complete separation of two rotamers was achieved at rather higher column temperatures. These results suggest that internal rotation about the carbonyl–nitrogen bond is seriously restricted when both *o*-positions of the benzene ring are occupied by bulky substituents.

Among the various acetanilides tested, only the following did not give the separation of the two rotamers; 2-nitroacetanilide, 3-nitroacetanilide, 4-nitroacetanilide, and *N*-methylacetanilide. This does not necessary mean that the content of the *E*-form is too small to be detected by HPLC, and the following alternative explanations are also possible. (1) The retention times of the two rotamers may happen to be quite similar for these acetanilides under the present HPLC conditions. (2) If the rate of interconversion between the two rotamers is much faster than the rate of separation by HPLC, the two rotamers will not be

Table 1. Percentages of acetanilides existing as E-form

Acetanilides	E-form/%			
	This work* (HPLC)	Other works		
		(NMR)	Experimental conditions	Ref.
2-Methylacetanilide	18	6	$-30\,°C$, $0.3\ \mathrm{mol\,dm^{-3}}$ in $CDCl_3$	8
2-Ethylacetanilide	24	15	$-20\,°C$, $0.3\ \mathrm{mol\,dm^{-3}}$ in $CDCl_3$	7
2-Isopropylacetanilide	29	25	$-20\,°C$, $0.3\ \mathrm{mol\,dm^{-3}}$ in $CDCl_3$	8
2,4-Dimethylacetanilide	25			
2,6-Dimethylacetanilide	31	26	$37\,°C$, $0.3\ \mathrm{mol\,dm^{-3}}$ in $CDCl_3$	8
		20	room temperature, 2% in CCl_4	20
2,4,6-Trimethylacetanilide	34			
3-Methylacetanilide	2.6			
4-Methylacetanilide	4.0			
Acetanilide	1.9	<1.0	$-20\,°C$, $0.3\ \mathrm{mol\,dm^{-3}}$ in $CDCl_3$	8
		0.1	room temperature, in pyridine	21
2-Hydroxyacetanilide	4.7			
3-Hydroxyacetanilide	1.6			
4-Hydroxyacetanilide	7.3			
4-Ethoxyacetanilide	6.1			

* 25 °C, 0.5% in $CHCl_3$.

Table 2. Percentages of E-forms of 2-methylacetanilide, 3-methylacetanilide, 4-methylacetanilide, and acetanilide in different solvents

	2-Methyl-acetanilide	3-Methyl-acetanilide	4-Methyl-acetanilide	Acetanilide
Solvent	E-form/%			
Hexane*	19	2.7	4.1	1.8
Carbon tetrachloride*	18	2.5	4.1	1.7
Benzene	18	2.5	3.8	1.9
Chloroform	18	2.6	4.0	1.9
Diethyl ether	17	2.1	3.7	1.6
1,4-Dioxane	18	2.2	3.7	1.1
Ethyl acetate	17	2.1	3.5	1.5
Acetonitrile	17	1.9	2.8	1.1
Ethanol	16	1.6	2.3	0.7
Methanol	15	1.5	2.1	0.6

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

separated. This phenomenon may occur especially in nitro-substituted acetanilides because the strongly electron-withdrawing nitro group in the benzene ring should reduce the double-bond character of the carbonyl–nitrogen bond. Actually, a decrease in the rotational energy barrier was observed for nitro-substituted formanilides.[10)]

The Ratios of the Two Rotamers. Various acetanilides were dissolved in chloroform (0.5%, 25 °C), and the ratios of the two rotamers were determined under the conditions where the complete separation is achieved. The results are summarized in Table 1. The content of the E-form was found to increase as the *o*-alkyl group becomes bulkier for *o*-alkyl-substituted acetanilides.

In order to examine the effect of the solvent on the ratios of the two rotamers, 2-methylacetanilide, 3-methylacetanilide, 4-methylacetanilide, and acetanilide were dissolved in a variety of solvents, and the ratios of the two rotamers were determined; the results are shown in Table 2. The population of the E-form was found to decrease in the following order; 2-methylacetanilide>4-methylacetanilide>3-methylacetanilide>acetanilide. Table 2 also shows that the population of the E-form is high when acetanilides are dissolved in nonpolar solvents, such as chloroform and hexane.

The effect of the concentration was examined on 2-methylacetanilide in chloroform. In contrast to formanilide,[3)] the percentage of the E-form is insensitive to the concentration. The contents of the E-form were calculated to be 18.0, 18.1, and 18.4% at 0.27, 1.1, and 8.6% in molar ratio, respectively.

NMR Measurements. The Proton NMR data hitherto reported have shown that a small amount of the E-form exists in the equilibrium state for *o*-substituted acetanilides, while for other acetanilides the presence of the E-form has not been confirmed by NMR. When we measured the ^1H-NMR spectra of 2-methylacetanilide, 3-methylacetanilide, 4-methylacetanilide, 2-ethylacetanilide, 2-isopropylacetanilide, and acetanilide carefully on a high-field NMR instrument, the following facts were found. A couple of signals corresponding to the E- and Z-rotamers

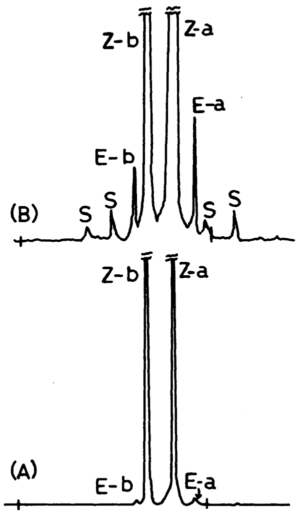


Fig. 3. The ^{1}H-NMR spectra of 3-methylacetanilide at 0 °C.
Sensitivity: (a) ×1, (b) ×5. E: *E*-form, Z: *Z*-form. a: Acetyl protons, b: methyl protons attached on benzene ring. S: spinning side bands.

Table 3. Comparison of NMR and HPLC data on the ratios of *E*-rotamers of acetanilides

	HPLC(25 °C) *E*-form/%	NMR *E*-form/%
2-Methylacetanilide	18	13[a]
3-Methylacetanilide	2.6	2.0[b]
4-Methylacetanilide	4.0	3.2[b]
Acetanilide	1.9	1.6[b]

2% solution. a) 10 °C, b) 0 °C.

appeared for *o*-substituted acetanilides at room temperature. For 4-methylacetanilide, two broad signals were observed at 10 °C; the larger one, at δ 2.15, should be assigned to the acetyl protons of the *Z*-form while the smaller one, at δ 2.05, should possibly corresponds to those of the *E*-form. The decrease in the temperature sharpened both signals (Fig. 3). On the other hand, when the temperature was increased, these signals were broadened; indeed, the smaller one was not recognized at 25 °C. At this temperature, these two signals probably coalesce. Thus, this phenomenon may be interpreted in terms of the interconversion between *E*- and *Z*-forms. 3-Methylacetanilide and acetanilide gave similar results. The percentage of the *E*-form of these acetanilides amounts to only 1—3%. These results are summarized in Table 3, together with the data obtained by HPLC. The two sets of results agree quite well. The NMR data hitherto reported by other authors,[7,8] however, give a smaller content of the *E*-form. We consider that the discrepancy between those results and ours may be interpreted in terms of the low sensitivity of the earlier, relatively low-field NMR instruments, for example, 60 MHz for ^{1}H. When NMR signals are broad and when, in addition, the signal-to-noise ratio is not good, the minor components are apt to be overlooked or sometimes estimated to be too small.

Discussion

The following facts seem noteworthy in considering the rotamer equilibrium of acetanilides in solution: (1) The presence of a considerable amount of the *E*-form in *o*-substituted acetanilides was confirmed by HPLC; this finding was in good agreement with the NMR data hitherto reported. (2) The presence of a very small amount of the *E*-form in several *o*-unsubstituted acetanilides was confirmed for the first time by HPLC. We examined the ^{1}H-NMR spectra of these anilides carefully and found small peaks which were attributable to the *E*-form. Since the content of *E*-form of acetanilide is very low, its detection has not been possible by several spectroscopic methods including NMR. Thus, the studies carried out hitherto by means of ^{1}H-NMR,[8] ^{13}C-NMR,[11] and photoelectron spectroscopy,[12] which has been considered to be a powerful tool for investigating labile equilibria in recent years, have failed to detect the *E*-form. (3) When a hydroxyl group was introduced into the benzene ring, the ratios of the *E*- and *Z*-rotamers were found to be different from those for the corresponding methyl-substituted acetanilides. The following sequence was found for increasing percentages of the *E*-form; 3-hydroxyacetanilide<2-hydroxyacetanilide<4-hydroxyacetanilide. The low percentage of the *E*-form of 2-hydroxyacetanilide may be explained in terms of some intramolecular hydrogen bonding in the *Z*-form.[13—15] (4) The ratios of the *E*- to the *Z*-form are somewhat dependent on the solvents. The percentage of the *E*-form is slightly higher in less polar solvents, although the effect is not so remarkable as that found for formanilide.[3] Furthermore, contrary to formanilide, the ratios of the two forms of 2-methylacetanilide in chloroform were almost unaffected by the concentration. Thus, the ring-dimer formation[16,17] attributable to intermolecular hydrogen bonding does not occur for acetanilides.

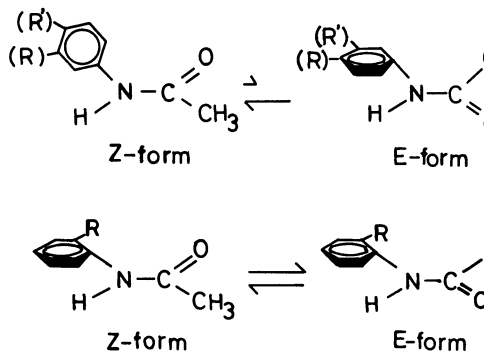
The acetyl protons of the *E*-rotamer appear at a higher field than those of the *Z*-rotamer (Table 4) in the ^{1}H-NMR spectra of not only *o*-substituted acetanilides, but also of other acetanilides. These high-field shifts may be due to the ring-current effect of the benzene ring, which suggests a twisted structure of the *E*-rotamer of these acetanilides. A previous ^{1}H-NMR study showed[8] that the *Z*-form of

TABLE 4. ^{1}H-NMR CHEMICAL SHIFTS OF THE ACETYL PROTONS

	E-form δ	Z-form δ
2-Methylacetanilide[a]	1.90	2.20
3-Methylacetanilide[b]	2.01	2.17
4-Methylacetanilide[b]	2.02	2.16
Acetanilide[b]	2.06	2.17

2% solution. a) 10 °C, b) 0 °C.

o-substituted acetanilides has a twisted structure. Contrary to this, the *Z*-forms of the non-*o*-substituted acetanilides have an almost planar structure,[8,18] although the X-ray data of acetanilide indicated[19] that, in the crystalline state, the plane of the aromatic ring deviates slightly from that of the amide group. On the base of these results, the rotamer equilibrium of acetanilides may be described as follows:



Both *E*- and *Z*-forms of *o*-substituted acetanilides have a twisted structure, because the *o*-substituted benzene ring causes a large steric hindrance if it is located coplanar with the amide plane. Contrary to this, the benzene ring of *o*-unsubstituted acetanilides will cause less steric hindrance. Thus, the *Z*-forms of these acetanilides may allowed to have an almost planar structure. The *E*-forms of these acetanilides, however, cannot have a planar structure, for a methyl group is bulkier than a carbonyl oxygen and the *E*-form will be more crowded than the *Z*-form. In the planar structure of the *Z*-form, since π-electrons in the benzene ring will get delocalized to some extent, a partially extended conjugated system will be formed. Thus, the *Z*-form will be more favoured energetically than the twisted *E*-form. The differences in free energies between the two rotamers ($\Delta G^\circ = -RT\ln K$, $K = [Z\text{-form}]/[E\text{-form}]$) are calculated to be -3.8 and $-7.9\ \mathrm{kJmol^{-1}}$ for 2-methylacetanilide and 4-methylacetanilide respectively, both in 0.5% solutions in chloroform at 25 °C. The difference between the two ΔG° values ($=4.1\ \mathrm{kJmol^{-1}}$) might be attributed to the difference in resonance energy between the *Z*-forms of the two acetanilides, provided that there is little difference in steric hindrance between the *E*-forms of these two acetanilides.

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