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Kinetic Studies of Fast Equilibrium by Means of High-Performance Liquid Chromatography. XIII.[1] Separation of Rotamers of *N*-Alkyl-Substituted Amides

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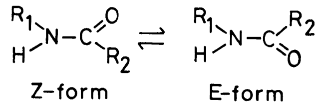
Synopsis. Rotamers of *N*-alkyl-substituted amides have been separated by means of high-performance liquid chromatography(HPLC) at low column temperatures. The ratios of the *E*-rotamers have been found to increase in the following order: propionamides<acetamides<formamides.

We have already reported[1–3] the separation of rotamers of anilides by means of HPLC at low column temperatures. A similar separation seems to be possible for aliphatic amides. This report deals with the separation of rotamers of *N*-alkyl-substituted amides. The separation is found to be rather difficult for the following reasons, although the energy barriers of the bond rotation about the carbonyl–nitrogen bonds for aliphatic amides are higher than those for anilides.[4] (1) Since aliphatic amides usally have only weak UV absorption in the range shorter than 230 nm, their detection is not easy when the amount is low. Furthermore, the solvent systems usable in HPLC is seriously restricted. (2) Aliphatic amides, especially their low molecular weight analogues such as *N*-methylformamide and *N*-methylacetamide, are adsorbed strongly on the surface of silica gel, and therefore broad and tailing peaks appear on the chromatograms.



Chart

Experimental

N-Methylformamide, *N*-methylacetamide, *N*-ethylacetamide, *N*-benzylformamide, and *N*-benzylacetamide were obtained commercially. *N*-Ethylformamide, *N*-propylformamide, and *N*-isopropylformamide were prepared from trichloroacetaldehyde (chloral) and the corresponding aliphatic amines.[5] *N*-Propylacetamide, *N*-isopropylacetamide, *N*-methylpropionamide, *N*-ethylpropionamide, and *N*-propylpropionamide were prepared by the reaction of acetamide (or propionamide) with hydrochlorides of aliphatic amines.[6] These synthesized amides were all purified by distillation. Several silica gel packings with different adsorptive activity (LiChrosorb SI 60, LiChrosorb SI 100, LiChrosphere SI 1000, and LiChrosphere SI 4000) were tried, and the least active among them (LiChrosphere SI 4000) gave the best results. Other experimental conditions were detailed elswhere.[1–3]

Results and Discussion

We first attempted the separation of rotamers of *N*-benzylformamide because this aliphatic amide has relatively strong absorption in the UV by dint of the benzyl substituent and the presence of both *E*-and *Z*-rotamers had been confirmed by NMR study.[7] The solvent system of hexane and 1-propanol containing a small amount of acetic acid, which had been successfully applied for the separation of rotamers of acetanilides,[3] did not give the separation of rotamers at different column temperatures (25 to −50 °C). The replacement of 1-propanol by other alcohols also did not give satisfactory results. The addition of acetic acid to the eluent had a significant effect on the peak shape and very broad and tailing peaks were obtained in the complete absence of acid. The increase of the acetic acid content suppressed tailing, but the two rotamers were still not separated. Addition of too much acetic acid is not suitable for UV detection due to the UV absorption of acetic acid. The activity of silica gel packings also had significant effects on the peak shape and the less active packings gave the better results. Other various solvent systems were tested for the separation of the rotamers of *N*-benzylformamide. The solvent system of hexane and ethyl acetate containing a small amount of acetic acid[2] achieved the separation of the rotamers at 0 °C, but this solvent system could not be used for other aliphatic amides due to the UV absorption of ethyl acetate. The use of other various common solvents including esters, ketones, and alkyl halides, was also impossible. The only solvent system which separated the rotamers was a mixture of hexane and diethyl ether containing a very small amount of acetic acid. Under this solvent system rotamers of *N*-alkyl-substituted amides including *N*-benzylformamide were separated as is exemplified in Fig. 1. The separation of formamides was achieved near 0 °C, while for the separation of acetamides a lower column temperature (−20 °C) was required. With the increase of the column temperature the two peaks

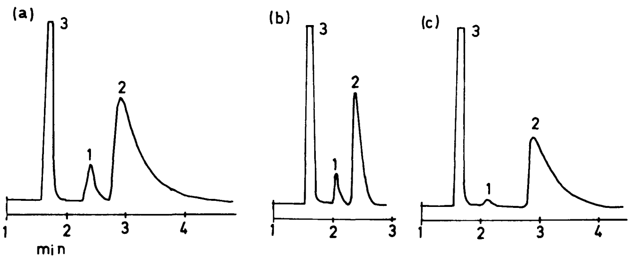


Fig. 1. Separation of rotamers of formamides and acetamides. Column: LiChrosphere SI 4000 (10 μm, 4.6 mm × 15 cm). Eluent : hexane : diethyl ethyl : acetic acid = 100 : 50 : 0.5, 2.5 cm^3 min^{-1}. Detector: UV 228 nm. Column temperature: (a) and (b) 0 °C, (c) −20 °C. (a): 0.2 mol dm^{-3} *N*-methylformamide, (b): 0.2 mol dm^{-3} *N*-benzylformamide, (c): 0.2 mol dm^{-3} *N*-ethylacetamide.
1: *E*-form, 2: *Z*-form, 3: solvent ($CHCl_3$).

Table 1. Percentages of Formamides and Acetamides Existing as *E*-Form

Amides	*E*-form/%			
	This work[a] (HPLC)	Other works		
		(NMR)	Experimental conditions	Ref.
N-Methylformamide	11	8	35 °C, pure liquid?[b]	8
N-Ethylformamide	19	12	35 °C, pure liquid?[b]	8
N-Propylformamide	22			
N-Isopropylformamide	21	12	35 °C, pure liquid?[b]	8
N-Benzylformamide	14	10	room temperature, 16.7% in molar ratio in benzene	7
N-Methylacetamide	2.0			
N-Ethylacetamide	2.1			
N-Propylacetamide	3.9			
N-Isopropylacetamide	2.0			
N-Benzylacetamide	1.1			

a) 25 °C, 0.2 mol dm^{-3}. b) Experimental conditions were not well documented.

coalesced gradually and at room temperature only one peak appeared. As shown in Fig. 1 the HPLC conditions are far from ideal due to tailing. Further addition of acetic acid would alleviate tailing, but this was impossible for the UV detection. Contrary to formamides and acetamides, *E*-rotamers of propionamides were not detected even at very low column temperatures (−60 °C), which suggests that either the content of the *E*-rotamers is very low (<0.5%) or retention times of the two rotamers are close to each other. We consider that the former explanation is more probable. Table 1 indicates the percentages of *E*-rotamers in chloroform. The identification of the peak was carried out by comparing the HPLC results with the data obtained by ^{1}H-NMR for formamides.[8] The presence of *E*-rotamers of acetamides had not hitherto been reported by NMR studies,[4] but the presence of a few percent of *E*-rotamers was confirmed by HPLC. The following sequence of increasing percentages of *E*-rotamers (propionamides<acetamides<formamides) is explained in terms of the difference in steric hindrance between R_1 and R_2 in the *E*-forms.

The ratios of rotamers should also be affected by various conditions such as concentration, temperature, and solvent composition in which the amides are dissolved. *N*-Methylformamide was dissolved in several solvents (0.2 mol dm^{-3}, 25 °C) and the percentages of *E*-rotamers were determined which were as follows; 11% (carbon tetrachloride), 11% (chloroform), 10% (benzene), 9.0% (tetrahydrofuran), 8.2% (ethyl acetate), 8.2% (acetonitrile), 7.0% (diethyl ether), 6.3% (ethanol), and 5.8% (methanol). The effect of the concentration was also examined for *N*-methylformamide in chloroform, and the percentages of the *E*-rotamer were as follows; 13% (0.13 mol dm^{-3}), 11% (0.67 mol dm^{-3}), and 7.8% (6.7 mol dm^{-3}). The population ratios were relatively insensitive to the solvent composition and concentration, although some tendency was observed for the content of the *E*-rotamer to be slightly higher in nonpolar solvents and in dilute solutions. Similar behavior was also observed for other formamides such as *N*-ethylformamide and *N*-benzylformamide. This behavior is different from that of formanilides because the population ratios of formanilides are sensitive to the solvent composition as well as to the concentration[2] due to ring dimer formation.[9] The population ratios of formamides have more resemblance to those of *o*-substituted acetanilides.[3] These results suggest that ring dimer formation does not play an important role in the case of formamides. The population ratios of acetamides, on the other hand, resemble those of *o*-unsubstituted acetanilides.

The attempt to determine the rotational energy barriers by means of HPLC[1] was not carried out due to tailing. It can however, be safely concluded that the energy barriers for aliphatic amides are larger than those for anilides, because the separation of the rotamers of aliphatic amides was possible at relatively higher column temperatures. It can also be concluded that the energy barriers to the bond rotation of formamides are higher than those of acetamides.

References

1) M. Moriyasu, K. Kawanishi, A. Kato, Y. Hashimoto, M. Sugiura, and T. Sai, *Bull. Chem. Soc. Jpn.*, **58**, 3351 (1985).

2) M. Moriyasu, K. Kawanishi, A. Kato, and Y. Hashimoto, *Bull. Chem. Soc. Jpn.*, **57**, 1766 (1984).

3) M. Moriyasu, K. Kawanishi, A. Kato, Y. Hashimoto, and M. Sugiura, *Bull. Chem. Soc. Jpn.*, **58**, 2581 (1985).

4) For the NMR study of the restricted bond rotation of various amides, see the following review and the references cited therein: W. E. Stewart and T. H. Siddall. III., *Chem. Rev.*, **70**, 517 (1970).

5) F. F. Blicke and C. Lu, *J. Am. Chem. Soc.*, **74**, 3933 (1952).

6) A. Galat and G. Elion, *J. Am. Chem. Soc.*, **65**, 1566 (1943).

7) W. Walter and G. Maerten, *Liebigs Ann. Chem.*, **712**, 58 (1968).

8) L. A. LaPlanche and M. T. Rogers, *J. Am. Chem. Soc.*, **86**, 337 (1964).

9) A. J. R. Bourn, D. G. Gillies, and E. W. Randall, *Tetrhedron*, **20**, 1811 (1964).