

Effect of Molecular Conformation on Interaction of Organic Species in Water. Benzamides and Anisamides with Theophylline and Riboflavin

By MASAHIRO NAKANO* and TAKERU HIGUCHI†

Complexing tendencies of *N,N*-dimethylbenzamide and *N,N*-dimethylanisamide toward theophylline and riboflavin in water were found to be significantly less than those of *N*-substituted and *N*-monomethyl analogs. It is suggested that rotation of *N,N*-dimethylamide group about the benzene ring-carbonyl carbon bond may inhibit formation of such complexes. *N,N*-Dimethylcinnamamide whose *N,N*-dimethylamide group can lie on the same plane as the styrene group was shown to be as good an interactant as the unsubstituted cinnamamide.

HIGUCHI AND PISANO (1) and Nakano with Higuchi (2) have observed that some *ortho*-substituted benzoate ions other than hydroxy were generally much weaker binding agents than the unsubstituted and the corresponding *meta*- or *para*-substituted benzoate ions. In these systems the observed effect was attributed to steric interferences by groups in the *ortho* position.

The present report is concerned with a similar observation resulting from a substitution in a side chain. *N,N*-Dimethylbenzamide was found to be a significantly weaker interactant toward theophylline and riboflavin than unsubstituted and *N*-monosubstituted benzamide. Measurements were also carried out on anisamides and cinnamamides.

EXPERIMENTAL

Materials—Benzamide (Eastman), anisamide (Aldrich), and cinnamamide (Aldrich) were recrystallized from water, m.p. 128°, 166°, 147°, respectively. *N*-Methylbenzamide, *N*-methylanisamide, *N,N*-dimethylbenzamide, *N,N*-dimethylanisamide, and *N,N*-dimethylcinnamamide were synthesized from their acid chlorides (Aldrich) and gaseous monomethylamine or dimethylamine in ether and purified by vacuum distillation or by recrystallization from water, m.p. 80°, m.p. 116°, b.p. 132° at 15 mm., m.p. 42°, and m.p. 103°, respectively. The PMR spectrum of each compound was also checked on a Varian A-60A spectrometer. Theophylline (m.p. 272°) and riboflavin were of NF and USP grade, respectively, and were used without further purification.

Solubility Studies—For benzamide-theophylline systems, increasing amounts of benzamide solution ($0.3 \times 10^{-1} M$) were pipetted into vials and the solutions were brought to a constant final volume (10 ml.) in presence of fixed, excess amount of solid theophylline. For cinnamamide-theophylline systems, increasing amounts of theophylline solution ($0.3 \times 10^{-2} M$) were pipetted into vials and excess amounts of cinnamamides were added. Equili-

bration was effected by rotation of the vials in a water bath thermostated at $25.0 \pm 0.1^\circ$ for 48 hr.

After equilibration, an aliquot of the supernatant liquid was withdrawn and analyzed spectrophotometrically after dilution with methanol. Wavelengths chosen were 282 m μ for benzamide-theophylline systems and 290 m μ for cinnamamide-theophylline systems. Small absorption due to the known amount of benzamides for the former systems and that of theophylline for the latter systems were subtracted from the total observed absorbance. Apparent 1:1 stability constants were computed according to the phase-solubility technique (3).

Spectrophotometric Studies—Stability constants for riboflavin complexes were evaluated from spectroscopic measurements. Volumetric flasks, each containing $4 \times 10^{-6} M$ riboflavin and varying amount of benzamides or anisamides ($0.5 \times 10^{-2} M$), were equilibrated in a 25° water bath. These solutions were brought to final equilibrium in a Cary 15 spectrophotometer cell compartment kept at 25°. The absorbance was measured at 490 m μ and the Foster *et al.* method (4) was employed for the computation of stability constants.

PMR Studies—Proton magnetic resonance (PMR) spectrum of *N,N*-dimethylbenzamide ($5 \times 10^{-2} M$) in water at 25° was recorded on a Varian A-60A spectrometer with a V-6057 variable temperature accessory. PMR spectra of anisamides ($3 \times 10^{-2} M$) in deuterium oxide were recorded on the same spectrometer at temperature of the probe (38°). DSS was used as an internal reference.

RESULTS AND DISCUSSION

Experimentally observed apparent stability constants of benzamide and substituted benzamides interacting with theophylline as determined by the solubility method are shown in Table I. It is probable that these values reflect, to a limited degree, contributions from species other than 1:1;

TABLE I—APPARENT 1:1 STABILITY CONSTANTS (M^{-1}) WITH THEOPHYLLINE IN WATER AT 25° DETERMINED BY THE SOLUBILITY METHOD

Compd.	Stability Constant (M^{-1})
Benzamide	12
<i>N</i> -Methylbenzamide	13
<i>N,N</i> -Dimethylbenzamide	2

Received July 10, 1967, from the School of Pharmacy, University of Wisconsin, Madison, WI 53706
Accepted for publication August 9, 1967.

M. Nakano expresses his appreciation to Professors K. Kakemi and H. Sezaki, Kyoto University, for their encouragement and interest.

This work was supported in part by grant GM-05830 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

* Present address: Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

† Present address: School of Pharmacy, University of Kansas, Lawrence, KS 66045 Address all correspondence to this address.

but these are not expected to affect significantly the relative values. Corresponding values obtained spectrophotometrically for a series of amides interacting with riboflavin are shown in Table II. For

TABLE II—APPARENT 1:1 STABILITY CONSTANTS (M^{-1}) WITH RIBOFLAVIN IN WATER AT 25° DETERMINED BY THE SPECTROPHOTOMETRIC METHOD

Compd.	Stability Constant (M^{-1})
Benzamide	8
<i>N</i> -Methylbenzamide	10
<i>N,N</i> -Dimethylbenzamide	2
Anisamide	21
<i>N</i> -Methylanisamide	33
<i>N,N</i> -Dimethylanisamide	4

both systems it would appear that a single *N* substitution produced a small but a definite increase in binding tendencies. *N,N*-Dimethyl series, however, exhibit in every case a dramatic drop-off in their associative ability. It is suggested that this situation may arise as a result of the difference in the steric conformation of the disubstituted amides as compared to the others. Benzamides and *N*-methylbenzamides can have completely planar structures which would permit maximum conjugation between the amide groupings and the benzene rings. These have, as expected, closely similar spectra (5). With *N,N*-dimethylbenzamides, on the other hand, a completely planar structure is impossible because of the interference between the methyl groups and the *ortho* hydrogen atoms (5). The interference can be relieved by rotation about the Ph—CO bond or the CO—NMe₂ bond. It is more likely that the amide group remains planar because of its high resonance energy (6), and that rotation takes place about the Ph—CO bond. The ultraviolet spectrum of *N,N*-dimethylbenzamide in water, furthermore, has no peak above 220 mμ, a situation characteristic of nonplanar molecules (7). The PMR spectrum of *N,N*-dimethylbenzamide at 25° in water, moreover, was observed to have 2 peaks, separated by 7 c.p.s. from each other, corresponding to 2 different methyl groups with respect to carbonyl group. This effect is most likely due to hindered rotation about the CO—NMe₂ bond. The

relative PMR chemical shifts of the *ortho* and *meta* hydrogens in anisamides shown in Fig. 1 also appear to reflect the effects of *N* methylation.

The complexing tendency toward theophylline of *N,N*-dimethylcinnamamide, whose amide group is sufficiently removed from the benzene ring for steric hindrance to planarity to be absent, was also examined and the results are shown in Table III.

Unlike the benzamide *N,N*-dimethylcinnamamide

TABLE III—APPARENT 1:1 STABILITY CONSTANTS (M^{-1}) WITH THEOPHYLLINE IN WATER AT 25° DETERMINED BY THE SOLUBILITY METHOD

Compd.	Stability Constant (M^{-1})
Cinnamamide	32
<i>N,N</i> -Dimethylcinnamamide	30

appears to be almost as good an interactant as the unsubstituted cinnamamide and has essentially the same absorption as the latter when account is taken of the bathochromic effect of the methyl groups (5).

It is believed that the interactions of aromatic molecules in water are likely plane to plane stacking (2). If this is true, the observed poor complexing tendencies of *N,N*-dimethylbenzamides are possibly due to prevention of approaching molecules from coming closer to the aromatic ring. The decreased interactive tendencies of the dialkyl derivatives, on the other hand, may also arise in part from the reduction in resonance caused by the steric effect.

REFERENCES

- (1) Higuchi, T., and Pisano, F. D., *J. Pharm. Sci.*, **53**, 644(1964).
- (2) Nakano, M., Ph. D. Thesis, University of Wisconsin, Madison, Wis., 1967.
- (3) Higuchi, T., and Connors, K. A., in "Advances in Analytical Chemistry and Instrumentation," vol. 4, Reilley, C. N., ed., Interscience Publishers, Inc., New York, N. Y., 1965, pp. 144–148.
- (4) Foster, R., Hammick, D. L., and Wardley, A. A., *J. Chem. Soc.*, **1953**, 3817.
- (5) Edward, J. T., and Meacock, S. C. R., *Chem. Ind.* **1955**, 536.
- (6) Pauling, L., Corey, R. B., and Branson, H. R., *Proc. Natl. Acad. Sci., U. S.*, **37**, 205(1951).
- (7) Braude, E. A., and Waight, E. S., in "Progress in Stereochemistry," Klyne, W., ed., Butterworths, London, England, 1954, p. 126.

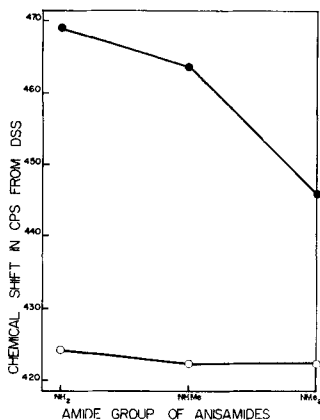


Fig. 1—The plots showing the chemical shifts of *ortho* (●) and *meta* (○) protons to amide group in anisamides (3×10^{-2} M) in D₂O.



Keyphrases

Molecular conformation effect on complex formation
Benzamide and substituted benzamide-theophylline systems
Cinnamamide and substituted cinnamamide-theophylline systems
Benzamide and substituted benzamide-riboflavin systems
Anisamide and substituted anisamide-riboflavin systems
PMR spectrometry
UV spectrophotometry—complex stability
Stability constants