greater than that of phenobarbital and barbital at the same conditions.

Partition coefficients have been measured between octanol–aqueous solution (9) and between erythrocyte membrane–buffer solution (10) for barbital, phenobarbital, and pentobarbital. With the assumptions that the numerical values of the interaction energies of barbital, phenobarbital, and pentobarbital are valid estimates (*i.e.*, the entropies are invariant) and that the same energies are operative for comparative purposes under the conditions in which partition coefficients were measured, the corresponding changes in free energies of transfer to the nonaqueous phase were calculated from literature data (9):

$$\Delta G_{tr} = RT \ln P \qquad \text{(Eq. 3)}$$

where P is the partition coefficient and ΔG_{tr} is the standard free energy associated with solute transfer from the aqueous to the nonaqueous phase. The order of such energies (ΔG_{tr}) (Table I) correlates with the interaction energies of barbital, phenobarbital, and pentobarbital with dipalmitoylphosphatidylethanolamine and with dipalmitoyllecithin monolayers and with their blocking concentrations.

These results show that the interaction energies of pentobarbital with the phospholipid monolayers are higher than those of barbital and phenobarbital and seem to indicate that its increased nerve blocking potency may be due to the comparatively greater interfacial concentration.

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Rationalization of Drug Complexation in Aqueous Solution by Use of Hückel Frontier Molecular Orbitals

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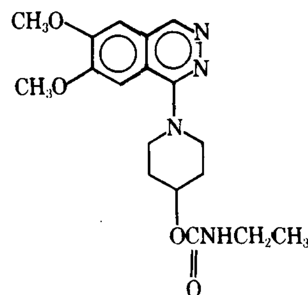
Received May 31, 1979, from *Pfizer Central Research, Sandwich, Kent, CT13 9NJ, England.* Accepted for publication July 25, 1979.

Abstract □ The complexation of certain drug molecules with niacinamide in aqueous solution was explained by the application of Hückel frontier molecular orbital calculations. A linear relationship was observed between the association constants derived from phase solubility studies and the interaction energy predicted by frontier molecular orbital calculations.

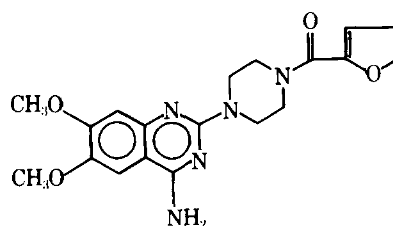
Keyphrases □ Niacinamide—complexation with drug molecules, aqueous solution, frontier molecular orbital calculations □ Complexation—of niacinamide with drug molecules, aqueous solution, frontier molecular orbital calculations □ Solubility—modification, niacinamide complexation with drug molecules, aqueous solution, frontier molecular orbital calculations

The solubility of drug substances often is modified by the use of additives. The discovery of the solubilizing (or solubility inhibiting) action of these additives frequently is made empirically; but in many cases, the system can be described by specific interactions between the drug and additive molecules. The use of phase solubility techniques to derive the association constants that quantitatively define the extent of interaction between the species involved was established (1). The nature of the specific interaction often is well understood.

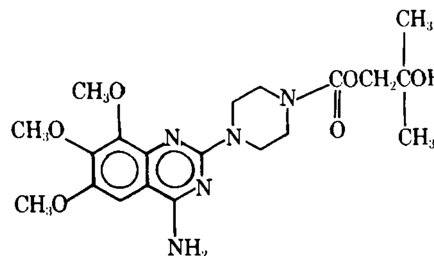
Many potential interactions can be exploited to modify drug solubility. The present work concerns the use of Hückel frontier molecular orbital (FMO) calculations to confirm a π-donor–π-acceptor mechanism for the interaction of 6,7-dimethoxy-1-[4-(ethylcarbamoyloxy)piperidino]phthalazine (I), 2-[4-(2-furoyl)piperazin-1-yl]-4-amino-6,7-dimethoxyquinazoline (II), 4-(4-amino-6,7,8-trimethoxyquinazolin-2-yl)piperazine-1-carboxylic acid 2-methyl-2-hydroxypropyl ester (III), and 6,7-dime-



I



II



III

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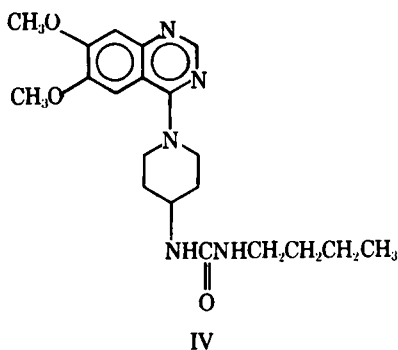

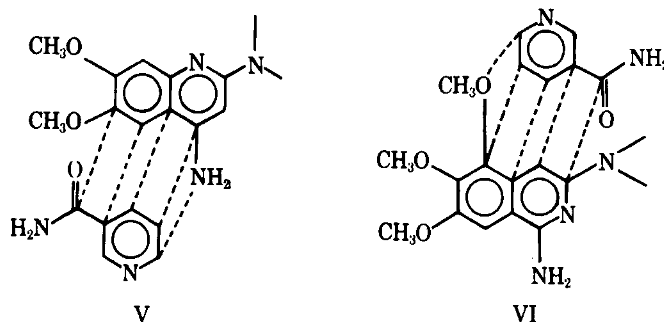

thoxy-4-[4-(3-*n*-butylureido)piperidino]quinazoline (IV) with niacinamide in aqueous solution. The association constants derived from phase solubility experiments were linearly related to the interaction energy predicted by frontier molecular orbital calculations.

EXPERIMENTAL

The phase solubility technique described previously (1) was used to investigate the influence of niacinamide on the solubility of Compounds I–IV. Aqueous niacinamide solutions of known concentration were equilibrated with the test compound by stirring at 25° for 24 hr so that a small excess of compound remained undissolved. The total concentration of the test compound in the filtered solutions was measured by UV spectrophotometry after dilution in methanolic hydrochloric acid. Suitable blanks were prepared from the niacinamide solutions.

RESULTS AND DISCUSSION

Association Constants—The phase solubility diagram of I–IV in niacinamide solution showed increased test compound solubility as the niacinamide concentration increased. The slight pH change accompanying the increased concentrations of niacinamide and the test compound did not explain the increased compound solubility since previous experiments showed the solubility of I–IV to be independent of pH in this region. The phase solubility diagrams all showed positive deviations from linearity. This behavior has been interpreted in terms of first- and second-order interactions between the compound (S) and the solubilizing agent (L):

$$S + L \rightleftharpoons SL$$

Scheme I

$$K_{1:1} = \frac{[SL]}{[S][L]} \quad \text{(Eq. 1)}$$

$$SL + L \rightleftharpoons SL_2$$

Scheme II

$$K_{1:2} = \frac{[SL_2]}{[SL][L]} \quad \text{(Eq. 2)}$$

The equations for the association constants $K_{1:1}$ and $K_{1:2}$ involve the free solubilizing agent concentration, $[L]$, in solution. Since only the total concentration of solubilizing agent can be defined, an iterative procedure was developed to isolate values for $K_{1:1}$ and $K_{1:2}$ (2). The values obtained by a similar iterative procedure are shown in Table I.

Molecular Orbital Calculations—The expected π-donor nature of the methoxy-substituted heteroaromatic centers in I–IV led to the choice of the π-acceptor niacinamide as a potential solubilizing agent. The remarkable successes of the simple Hückel frontier molecular orbital approach in rationalizing not only the classical cycloaddition reaction (3) but also numerous other organic reactions (4) encouraged the exploration of a frontier molecular orbital correlation based on the perturbation approach.

Table I—Equilibrium Constants and Calculated Stabilization Energies for Niacinamide [a] Complexes

Compound	$K_{1:1}$	$K_{1:2}$	ΔE	$\log K_{1:1}$	Calculated $\log K_{1:1}$
I	12.31	14.76	0.598	1.090	1.022
II	7.36	0.33	0.561	0.861	0.876
III	18.45	0.51	0.660	1.266	1.267
IV	9.88	2.26	0.607	0.995	1.058

[a] Niacinamide concentration was 0.0–2.0 M for II and 0.0–0.2 M otherwise.

In Eq. 3, ΔE is a partial measure of stabilization of the complex between a drug molecule S and the additive L (niacinamide in this case):

$$\Delta E = 2\gamma^2 \left[\frac{(\sum C_s^O C_l^U)^2}{E_s^O - E_l^U} + \frac{(\sum C_s^U C_l^O)^2}{E_l^O - E_s^U}\right] \quad \text{(Eq. 3)}$$

The ΔE value is analogous to the stabilization energy of the transition state between two reactants (5). Positions s of S and l of L are juxtaposed, and γ is the resonance integral for the interacting molecular orbital lobes at each site. The C and E values are eigenvectors and eigenvalues appropriate to the occupied (O) and unoccupied (U) interacting frontier orbitals. Consideration of secondary orbital overlap was necessary to rationalize certain cycloaddition reactions (6). Similarly, the highest and the next-to-highest occupied orbitals as well as the lowest unoccupied orbitals are included in the summation.

Several assumptions are inherent in the use of Eq. 3:

1. Electrostatic force variations are small compared with frontier molecular orbital interactions (7) so coulombic interaction is neglected.
2. Only the delocalized π-electron systems are involved in complexation.
3. Because the complexing agent is constant, changes in solvation from one complex to another can be considered constant.

In using Eq. 3, the overlap and orientation principle (8) is invoked, and it is considered that the ring systems of the two partners occupy parallel planes for complexes in water (9).

The program used to obtain molecular orbital indexes was developed previously (10). The coulombic and resonance values were obtained from the literature (11). The maximum ΔE value corresponds to the most favorable overlap of orbitals; V shows the most favorable overlap for I, II, and IV; and VI shows the most favorable overlap for the trimethoxy Compound III. The ΔE values obtained from Eq. 3 for these overlaps are shown in Table I.

Equation 4 gives the regression line for the $K_{1:1}$ association constants and the ΔE values for these four complexes ($n = 4$, $r = 0.95$, $p < 0.05$):

$$\log K_{1:1} = 3.95\,\Delta E - 1.34 \quad \text{(Eq. 4)}$$

This result is believed to justify the assumptions made and is sufficient to encourage further use of frontier molecular orbital theory in attempting to rationalize solution complexation.

The complexation of III shown in VI demonstrates that a simple change in substituent position can alter totally the complexation topology. The frontier molecular orbital theory applied to drug–receptor interactions could throw light on structure–activity relationships. Previously inexplicable "outliers" in regression analyses between biological activity and linear combinations of free energy-related parameters, as used in pioneering methods (12), now might be explained in terms of orbital symmetry if the receptor contains a conjugated π-electron system.

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Fluorescence Assay of Nitrofurantoin with *o*-Aminothiophenol in Plasma and Urine

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Received February 1, 1979, from the *School of Pharmaceutical Sciences, Showa University, Hatanodai, Shinagawa-ku, Tokyo, 142, Japan.* Accepted for publication August 1, 1979.

Abstract □ A fluorescence method is presented for the determination of nitrofurantoin based on conversion of the drug to a fluorescent substance. The method requires 0.1–0.5 ml of plasma or diluted urine and is 10 times more sensitive than the commonly used colorimetric method.

Keyphrases □ Nitrofurantoin—fluorescence assay, plasma and urine □ Spectrophotofluorometry—analysis, nitrofurantoin, plasma and urine □ Antibacterials—nitrofurantoin, fluorescence assay, plasma and urine

Nitrofurantoin, 1-(5-nitro-2-furfurylideneamino)hydantoin (I), is used to treat urinary tract infections. The determination of drug levels in body fluids is important for pharmacokinetic and bioavailability studies. The content of I in plasma and urine commonly is determined by a colorimetric method based on the formation of a hyamine complex of I after its extraction into nitromethane (1, 2).

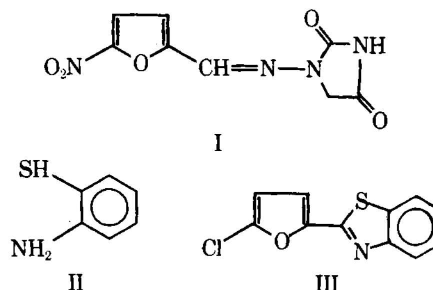
An electrochemical determination of I in urine that is faster and more accurate than the colorimetric method was reported (3). However, both methods require large sample volumes and are insufficiently sensitive for pharmacokinetic studies.

The reaction products obtained by heating some aromatic aldehydes with *o*-aminothiophenol (II) are fluorescent (4), and some drugs were assayed with II utilizing this reaction (5, 6). This paper discusses the application of this reaction to the fluorescence assay of I in plasma and urine.

EXPERIMENTAL

Reagents—Nitrofurantoin[1] (I) was obtained commercially. *o*-Aminothiophenol hydrochloride was prepared by recrystallization of crude crystals obtained from a mixture of 1 ml of hydrochloric acid/g of *o*-aminothiophenol[2] (II) in ethanol (4). The other reagents were reagent grade.

Procedure—Plasma, 0.1–0.5 ml, was deproteinized by mixing with 1 ml of ethanol. After centrifugation at 3000 rpm for 10 min, 0.5 ml of the supernate was transferred to a 15-ml test tube. Then 1 ml of 0.001% sodium nitrite, 2 ml of hydrochloric acid, 1 ml of 0.1% *o*-aminothiophenol hydrochloride, and 0.5 ml of distilled water were added. After mixing,



the solution was heated at 70° in a water bath for 10 min. After cooling to room temperature, UV irradiation was carried out for 3 min; the relative fluoresence intensity was measured with excitation and emission at 375 and 422 nm, respectively.

The content of I in urine was determined similarly. If the urine was diluted eightfold or more with distilled water, 1 ml of the diluted urine could be assayed directly without deproteinization.

The spectrophotofluorometer[3] was standardized with quinine sulfate[4] in 0.1 *N* H_2SO_4.

The concentration of I in a sample was determined by comparing its fluorescence intensity with that of a standard solution prepared by adding a known concentration of I dissolved in ethanol to plasma or urine free of I and processing it in the same way as the test sample.

RESULTS AND DISCUSSION

A fluorescent substance, 2-(5-chloro-2-furyl)benzothiazole (III), was formed by the reaction of the 2-furaldehyde obtained by hydrochloric acid hydrolysis of I with II (6). However, the conversion of I to III varied due to a steric effect of the side chain of I (7) and incomplete replacement of the nitro group of 2-(5-nitro-2-furyl)benzothiazole with chlorine to give III (6). The nitro group can be replaced with chlorine by UV irradiation (6). The hydrolysis of I is reversible and is promoted by sodium nitrite, acetylacetone, formaldehyde, *etc.*

The maximum relative intensity of fluorescence was obtained in the presence of 0.001% sodium nitrite and with UV irradiation after heating. Therefore, these conditions were adopted for the assay of I with II. Typical results are summarized in Table I.

A linear relationship was obtained between the relative fluorescence intensity and the I concentration up to 5 μg/ml. The means of the relative fluorescence intensity of plasma samples varied slightly within a given analysis, but the standard deviations were similar. For this reason, it is necessary to run a standard I solution with each plasma and urine analysis. Under these conditions, quantitative measurements are possible.

To check the fluorescence method, plasma and urine samples from

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[2] Tokyo Kasei Kogyo Co., Tokyo, Japan.
[3] Hitachi MPF-2A.
[4] Wako Pure Chemical Industries, Osaka, Japan.

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