ml), and the mixture was decanted. The lower layer was cooled to −12°, and benzylamine (12.2 g, 0.11 mole) was then added dropwise with stirring. The mixture was stirred at ambient temperature for 16 hr and filtered. The residue was dissolved in methanol and treated with activated charcoal, and the product was precipitated with isopropyl ether, 2.8 g (12.5% crude yield), mp 151–153°; IR (KBr): 2190 (cyano) and 1660 (amide) cm^{-1}.

Several attempts to isolate the product led to decomposition, as evidenced by the disappearance of IR absorption of the cyano group. Therefore, the product was used in the next step without further purification.

Reduction of IV—Crude IV (1.3 g, 0.005 mole) was suspended in anhydrous tetrahydrofuran (200 ml) and added dropwise to a solution of sodium bis(2-methoxyethoxy)aluminum hydride in benzene (10 ml, 0.034 mole). The mixture was heated at reflux temperature for 3.5 hr. After stirring at ambient temperature for 13 hr, excess hydride was decomposed by successively adding ethanol (95%, 4 ml) and water (6 ml). The supernate was separated and concentrated *in vacuo*.

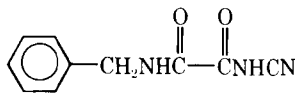
The oily residue was dissolved in chloroform (20 ml) and washed with water (3 × 10 ml). The dried chloroform solution (anhydrous magnesium sulfate) was evaporated *in vacuo*, and the residue was converted to a hydrochloride salt with acetone and concentrated hydrochloric acid. The product was recrystallized from absolute ethanol, 0.1 g (0.6% overall), mp 272–276°. Mixed melting-point determination with an authentic sample of III dihydrochloride showed no depression.

RESULTS AND DISCUSSION

In several repeat experiments, different workup procedures did not improve the yields of II and III. The components of the black tar, which make up the bulk of the crude yield, were not resolved.

Products II and III were identified as hydrochloride salts by microanalyses and mixed melting-point determinations with authentic samples. GLC analyses of the free bases provided confirming evidence.

Migration of a cyano group in a Beckmann-type rearrangement of I would be expected to yield IV as an intermediate. However, only starting



IV

material was recovered (90%) when I was treated under conditions known to cause a Beckmann rearrangement (7). When IV was treated under the same reducing conditions as I, only one product, III, was isolated. GLC analysis of the filtrate from the recrystallization of this reduction product failed to detect the presence of II.

Additional studies will be required to elucidate the rearrangement mechanism. However, sufficient evidence is presented to propose the migration of a cyano group.

REFERENCES

(1) M. R. Zimmermann, *J. Prakt. Chem.*, **66**, 353 (1902); through *Org. React.*, **11** (1960).

(2) O. C. Dermer and J. King, *J. Org. Chem.*, **8**, 168 (1943).

(3) E. Jucker and E. Rissi, *Helv. Chim. Acta*, **45**, 2383 (1962).

(4) S. C. Dickerman and A. J. Besozzi, *J. Org. Chem.*, **19**, 1855 (1954).

(5) R. A. Crochet, Jr., and C. D. Blanton, Jr., *Synthesis*, **1974**, 55.

(6) A. E. Kretov and A. P. Momsenko, *Zh. Org. Khim.*, **1**, 1734 (1965); through *Chem. Abstr.*, **64**, 3344 (1966).

(7) A. I. Vogel, "Practical Organic Chemistry," 3rd ed., Academic, New York, N.Y., 1956, p. 741.

ACKNOWLEDGMENTS

Abstracted in part from a thesis submitted by C. P. Chen to the School of Pharmacy, Auburn University, in partial fulfillment of the Master of Science degree requirements.

The technical assistance of Gloria Klase Ingram is gratefully acknowledged.

Interaction of Tricyclic Antipsychotic and Antidepressant Drugs with 1-Anilino-8-naphthalenesulfonic Acid

H. W. JUN ˣ and P. C. RUENITZ

Received November 15, 1976, from the *Department of Pharmacy, School of Pharmacy, University of Georgia, Athens, GA 30602.* Accepted for publication September 9, 1977.

Abstract □ The binding of 1-anilino-8-naphthalenesulfonic acid to selected tricyclic antipsychotic and antidepressant drugs was studied by fluorescence spectroscopy. The acid exhibited an increase in fluorescence intensity accompanied by a hypsochromic shift of the emission λ_{max} in the presence of these drugs. These fluorescence characteristics, in addition to those of acid–drug complexes after addition of potassium chloride or urea, suggested that binding was hydrophobic. The spectra also provided evidence regarding the importance of certain structural features of drugs in determining the nature of binding.

Keyphrases □ 1-Anilino-8-naphthalenesulfonic acid—binding to various antipsychotic and antidepressant drugs, fluorometric study □ Antipsychotics, various—binding to 1-anilino-8-naphthalenesulfonic acid, fluorometric study □ Antidepressants, various—binding to 1-anilino-8-naphthalenesulfonic acid, fluorometric study □ Binding—1-anilino-8-naphthalenesulfonic acid to various antipsychotics and antidepressants, fluorometric study □ Fluorometry—study of binding of 1-anilino-8-naphthalenesulfonic acid to various antipsychotics and antidepressants

Studies of molecular interactions in aqueous solution are important for elucidating the nature and mechanism of drug–protein binding in the body. Information pertaining to such interactions between small molecules can permit possible prediction of this drug–biomolecule binding. One technique commonly used is to measure spectral changes of drugs on binding to other molecules. Several reports (1, 2) concerned the use of absorption spectra for drug binding studies. Fluorescence spectroscopy (3) also was used to study molecular interactions.

Fluorescent probes, recently applied in drug–protein binding studies, are minimally fluorescent in their free form in aqueous solution. When these compounds are bound to other molecules, their spectral properties such as intensity and maximal emission wavelength change, reflecting their molecular environment. These spectral

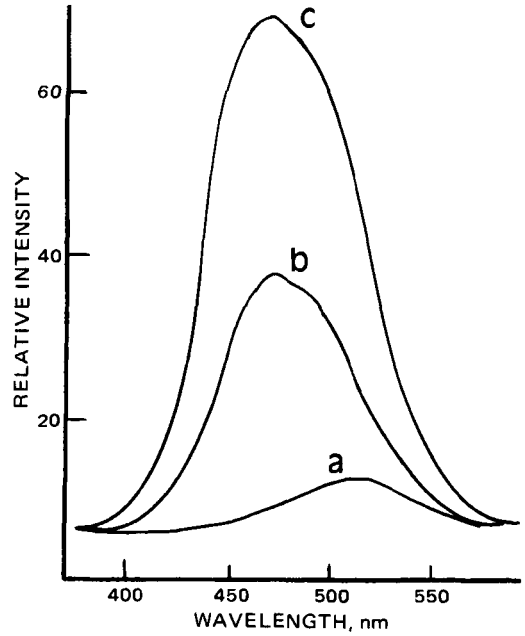


Figure 1—*Fluorescence emission spectra of the probe in pH 5.8 phosphate buffer (curve a) and on addition of trimeprazine (curve b) or chlorpromazine (curve c). The excitation maximum was set at 387 nm.*

changes have been measured to study their binding characteristics to enzymes (4), blood proteins (5), hormones (6), and blood cells (7). In particular, 1-anilino-8-naphthalenesulfonic acid (I) has often been used as a tool to study the site and nature of hydrophobic binding.

This paper reports a study of fluorescence spectral characteristics of I in the presence of several relatively small molecules, representing the dibenzazepine and phenothiazine classes of central nervous system active drugs.

EXPERIMENTAL

Materials—1-Anilino-8-naphthalenesulfonic acid[1] (I), chlorpromazine hydrochloride[2], trimeprazine tartrate[2], imipramine[3], and desipramine[3] were used. Spectroscopic grade methanol[4] was the solvent for the probe. All other chemicals were reagent grade. Water was double distilled from glass containers.

Apparatus—All fluorescence measurements were made on a spectrophotofluorometer[5] equipped with a 150-w xenon lamp and lp21 photomultiplier tube. Spectra were recorded with an x–y recorder[6].

Methods—Measurements were made in a fluorescence cell using 2-ml aliquots of aqueous solutions of imipramine, desipramine, chlorpromazine, and trimeprazine (all 2 mM). To each aliquot was added 5 μl of a 10 mM methanolic solution of I, and the fluorescence emission spectra were taken at 25 ± 1° at an excitation wavelength of 387 nm. Solutions of the drugs in the absence of added I exhibited negligible fluorescence spectra under the same experimental conditions.

RESULTS

Interaction of Chlorpromazine and Trimeprazine with I—Figure 1 shows emission spectra of I in water and in the presence of trimeprazine and chlorpromazine. Chlorpromazine caused a spectral shift in its emission maxima from 520 to 485 nm, accompanied by a large increase in fluorescence intensity (curve c). When chlorpromazine was present in large excess, the enhancement in fluorescence intensity as a function of I concentration was approximately linear (Fig. 2), suggesting a proportional increase in the fraction of the bound form of I in the system.

Table I—Influence of Potassium Chloride and Urea on the Relative Fluorescence of I–Chlorpromazine and I–Imipramine Complexes

Compounds Dissolved in Water	Emission Maxima, nm[a]	Relative Fluorescence
0.01 mM I	520	1
2 mM chlorpromazine	485	2
2 mM chlorpromazine, 0.01 mM I	485	30
2 mM chlorpromazine, 0.01 mM I, 3% KCl	485	100
2 mM chlorpromazine, 0.01 mM I, 2 M urea	485	6
2 mM imipramine	487	2
2 mM imipramine, 0.01 mM I	487	20
2 mM imipramine, 0.01 mM I, 3% KCl	487	70
2 mM imipramine, 0.01 mM I, 2 M urea	487	10

[a] The excitation maximum was 387 nm.

The presence of potassium chloride in the I–chlorpromazine system further increased fluorescence intensity. In contrast, addition of urea decreased fluorescence (Table I). The effects of potassium chloride and urea concentration on fluorescence intensity appeared concentration dependent.

A solution of I in the presence of trimeprazine exhibited less fluorescence increase compared to chlorpromazine (Fig. 1).

Interaction of Imipramine and Desipramine with I—Fluorescence spectra of I in water and in the presence of imipramine or desipramine are recorded in Fig. 3. Similar spectral changes were observed in the presence of each compound. When the concentration of free imipramine was high, the fluorescence intensity of the I–imipramine complex increased as a function of the I concentration (curve b, Fig. 2). The effects of urea and potassium chloride on fluorescence intensity of the I–imipramine complex were similar to those of the probe–chlorpromazine interaction (Table I).

DISCUSSION

Compound I and other fluorescent compounds interact with large biomolecules such as plasma proteins and enzymes and thus have served as probes in studies of small molecule–protein binding (4, 5, 8). Less attention, however, has been directed toward interactions of I with relatively small organic drug molecules. In this study, fluorescence spectral changes of I were found to be due to its hydrophobic interaction with the drug molecules in an aqueous solution.

A hypsochromic shift accompanied by an increased emission intensity was seen in the spectra of I in the presence of chlorpromazine, trimeprazine, imipramine, and desipramine. These spectral characteristics are indicative of interactions involving hydrophobic binding. Similar changes in the spectra of I due to hydrophobic interactions with biological macromolecules were established. When I was dissolved in nonpolar

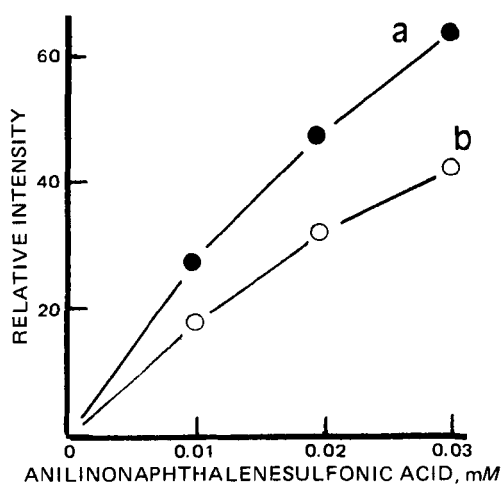


Figure 2—*Relative fluorescence intensity of I–chlorpromazine (curve a) and I–imipramine (curve b) interactions as a function of I concentration. The drug concentration was 2 × 10^{-3} M in water.*

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Smith Kline & French Laboratories, Philadelphia, Pa.
[3] Geigy Pharmaceuticals, Ardsley, N.Y.
[4] Matheson, Coleman & Bell, Norwood, Ohio.
[5] Aminco-Bowman, American Instrument Co., Silver Spring, Md.
[6] Omnigraphic, Houston Instruments, Bellaire, Tex.

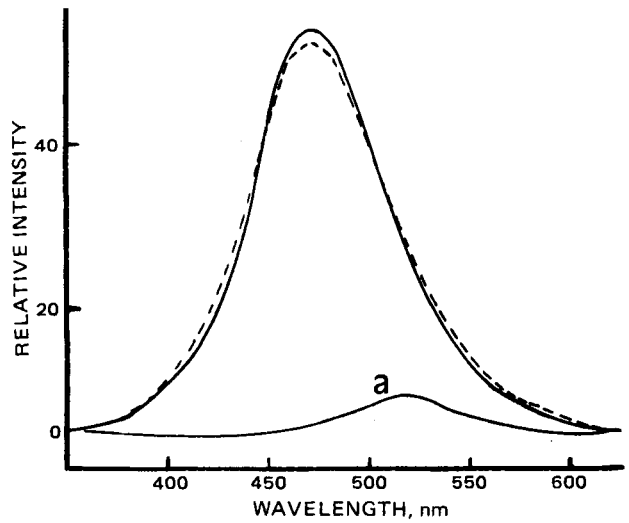


Figure 3—*Fluorescence emission spectra of the probe in pH 7.0 phosphate buffer (curve a) and when bound to imipramine (—) and to desipramine (- - -). The excitation maximum was set at 387 nm.*

solvents, it exhibited a large hypsochromic shift and increased fluorescence intensity (9–12). A direct correlation between the hydrophobicity of the solvent and the degree of spectral changes was found. It was suggested that hypsochromic shifts and enhanced fluorescence intensity were due to the conservation of excited energy in environments of decreasing polarity. The effects of potassium chloride and urea, which are known to bring about salting-out and salting-in effects, respectively, on the fluorescence properties of the I–chlorpromazine and I–imipramine complexes (Table I) provided additional evidence of the hydrophobic nature of the interaction.

Comparison of curves b and c in Fig. 1 suggested that chlorpromazine forms a stronger complex with I than does trimeprazine. The 2-chloro substituent in one aromatic ring of chlorpromazine appears to increase hydrophobic binding over the latter compound, which contains no chlorine substituents in its phenothiazine ring system. The greater ability of chlorpromazine to lower surface tension in aqueous solution relative to trimeprazine also was attributed to the greater hydrophobic effect of the 2-chlorophenothiazine ring of chlorpromazine (13). It was suggested that hydrophobic binding of these and related drugs to bovine serum albumin involved principally the phenothiazine moiety, with substituents attached to its nitrogen atom being of little significance in this regard (14). In accordance with this theory, the phenothiazine ring of chlorpromazine and, to a somewhat lesser extent, that of trimeprazine interact hydrophobically with the anilinonaphthalene ring of I, which causes the spectral changes.

The spectra of solutions of I in the presence of imipramine and desipramine exhibited similar emission spectra (Fig. 3). These compounds are structurally dissimilar only in the lack of a one side-chain methyl group in desipramine. Each contains a dibenzazepine ring system, which is presumably involved principally in hydrophobic binding with I, as in the case of the drugs containing a phenothiazine ring. This finding supports the suggestion (14) that side-chain substitution does not contribute to hydrophobic binding, since its participation in these otherwise structurally identical compounds would be expected to cause spectral differences.

REFERENCES

(1) J. L. Irvin and E. M. Irvin, *J. Biol. Chem.*, **196**, 651 (1952).

(2) C. F. Chignell, *Mol. Pharmacol.*, **5**, 244 (1969).

(3) D. A. Labianca, G. N. Taylor, and G. S. Hammond, *J. Am. Chem. Soc.*, **94**, 3679 (1972).

(4) L. Brand, J. R. Gohke, and D. S. Rao, *Biochemistry*, **6**, 3510 (1967).

(5) C. F. Chignell, *Mol. Pharmacol.*, **6**, 1 (1970).

(6) S. M. Aloj, K. C. Ingham, and H. Edelhoch, *Arch. Biochem. Biophys.*, **155**, 478 (1973).

(7) B. Rubalcava, D. M. Muñoz, and C. Gitler, *Biochemistry*, **8**, 2742 (1969).

(8) R. F. Chen and J. C. Kernohan, *J. Biol. Chem.*, **242**, 5813 (1967).

(9) L. Stryer, *J. Mol. Biol.*, **13**, 482 (1965).

(10) G. M. Edelman and W. O. McClure, *Acc. Chem. Res.*, **1**, 65 (1968).

(11) A. Hamabata, S. Chang, and P. H. von Hippel, *Biochemistry*, **12**, 1278 (1973).

(12) P. Mukerjee and K. J. Mysels, *Natl. Stand. Ref. Data Ser., Natl. Bur. Stand.*, No. 36 (1971).

(13) G. Zografi and M. V. Munshi, *J. Pharm. Sci.*, **59**, 819 (1970).

(14) J. Krieglstein, W. Meiler, and J. Stoob, *Biochem. Pharmacol.*, **21**, 985 (1972).

Synthesis of Antifungal 2-Substituted Phthalimidines

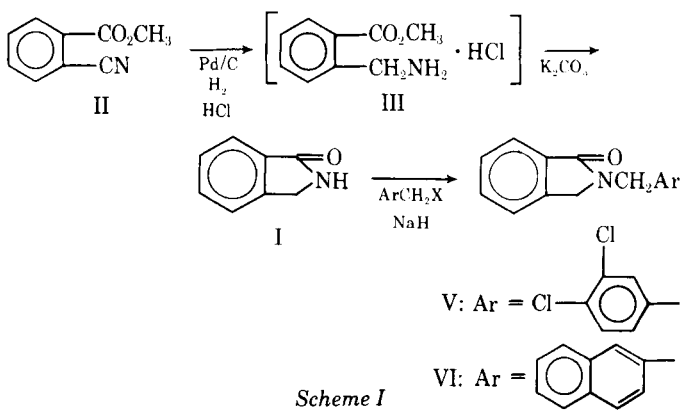
THOMAS J. SCHWAN ˣ **and JOSEPH E. GRAY**

Received August 19, 1977, from the *Research and Development Department, Norwich-Eaton Pharmaceuticals Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication September 16, 1977.

Abstract □ An improved synthesis of phthalimidine is reported. Two *N*-substituted phthalimidines were synthesized by alkylation of the parent compound, and they showed complete inhibition of *Microsporum* and *Trichophyton* species at 10–100 μg/ml in an agar dilution test. Both compounds were inactive against *Candida albicans* at 100 μg/ml or *Aspergillus niger* at 250 μg/ml.

Keyphrases □ Phthalimidines, substituted—synthesized, evaluated for antifungal activity □ Antifungal activity—various substituted phthalimidines evaluated □ Structure–activity relationships—various substituted phthalimidines evaluated for antifungal activity

Several *N*-substituted phthalimidines were desired for antifungal screening, so a convenient route to the parent compound I (Scheme I) was sought.



Scheme I