dergo side-chain oxidations. Oxidation occurs through the stages of benzyl methyl ketone and 1-phenyl-propane-2-ol to the acid. A corresponding mechanism would be expected to occur with fenfluramine. The amphetamines also undergo ring oxidation to give the phenol. The presence of the trifluoromethyl group on the benzene ring of fenfluramine appears to prevent such oxidation. The phenolic derivative of fenfluramine was not available to determine whether the reaction given by Axelrod would take place, but the recovery of 77 to 100% of the administered drug as fenfluramine and its two metabolites suggests that if such oxidation does occur, it can be only to a very small extent.

Previous observations (2, 5) have indicated that fenfluramine is rapidly absorbed into the tissues and is then slowly released into the extracellular fluid. This is also indicated by the present studies. The two amines continue to be excreted over at least a 72-hr. period and during the first 48 hr. *m*-trifluoromethylhippuric acid is excreted at an almost continuous rate.

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Keyphrases

Fenfluramine—metabolism
m-Trifluoromethylhippuric acid, de-ethylated fenfluramine—metabolites
Blood levels—fenfluramine
Urinary excretion—fenfluramine and metabolites
GLC—analysis

Structure-Activity Relations in an Imidazoline Series Prepared for Their Analgesic Properties

By W. BROCK NEELY*, H. C. WHITE†, and A. RUDZIK‡

A specific pharmacological property of a series of substituted imidazolines was examined in an effort to determine the structure-activity relationships that might be present. The best measure of the chemical reactivity was based on a charge-transfer complex between the drug and the receptor site. Using the molecular orbital technique for measuring this reactivity index, and combining with the partition coefficient in the manner described by Hansch 94.4 percent correlation was found for the biological activity.

STRUCTURE-ACTIVITY relationships have been an intriguing subject for organic and biological chemists for many years. At the same time, it is an area that has seen many frustrations and disappointments. The reasons for the failures are readily appreciated when the complexity of the biological system is taken into consideration. In attempting any type of rigid structure-activity study all of the the biological parameters such as active and passive transport, metabolism to a more or less active agent, nonspecific binding to proteins, and finally the reaction at the receptor site, must be taken into account. Obviously, the system is much too complicated and too little understood for a detailed analysis. As a starting point, a simple model is required. In such a case, if the model works, *i.e.*, can predict the data, it may give new insights into the mechanism of action. Conversely, if the model fails, then one must look for the reasons; this in itself could provide new concepts in the area under investigation.

The model used in this study is the one that has been developed by Hansch and his co-workers (1) and used extensively in a number of different biological applications (1–5). In effect, the model assumes that the amount of drug found at the receptor site is related to a single substituent constant which is derived from octanol–water partition-coefficient measurements. The reaction of the chemical at the receptor site is related to the rate constant. The model is illustrated by Eq. 1.

$$\log \text{B.R.} = -\alpha\pi^2 + b\pi + c \log k_x + d \qquad \text{(Eq. 1)}$$

where B.R. = biological response; π = log

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The authors thank R. Bidwell in the Technical Service and Development Group, The Dow Chemical Co., for his help in obtaining the oxidation potential data for the phenols reported in this paper. In addition, the technical assistance of D. V. Wysong and L. E. Begin is gratefully acknowledged for their help in the synthesis program.

(P_x/P_h); P_x, P_h = partition coefficient of substituted compound P_x and parent compound P_h; k_x = rate constant; a, b, c, d = equation constants.

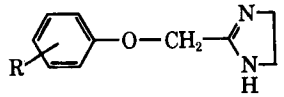
In attempting to arrive at some measure of the reactivity of a drug with a receptor site it is desirable to have some idea of what the mechanism might be. For example, in interpreting the insecticidal data of organophosphates, it is assumed that those phosphates which are most susceptible to nucleophilic attack will be the insecticidal agents. Consequently, a measure of the reactivity is the rate of hydrolysis of the phosphate by hydroxide ion. There are many papers that deal with this subject, but only a few are cited (6–8).

In the present case, the authors do not have a mechanism for the pharmacological activity (which in this case is assumed to be analgesic action); consequently, there is a problem of deciding on an appropriate reactivity index for the imidazoline compounds, I. What is required is a technique for estimating different electronic parameters and attempting to deduce which index correlates with the biological response. This requirement is met by the use of the simple Hückel molecular orbital (MO) technique. The theory and applications of MO to problems in biochemistry have been described by many authors (9–12). In using these electronic parameters as a measure of the rate constant in Eq. 1 the following assumption must be remembered. The rate constant k is related to the energy of activation E by means of the Arrhenius Eq. 2. The assumption

$$k = Ae^{-\Delta E/RT} \quad \text{(Eq. 2)}$$
$$\text{or } \log k = A - B\Delta E$$

where A and B are constants, is that the various indexes calculated from MO theory are linearly related to ΔE and hence may be substituted for $\log k$ in the regression equation, Eq. 1.

This paper reports the results in using the Hansch approach to correlate the pharmacologic activity, derived from an analgesic test, with the structure of a series of imidazoline derivatives, I.

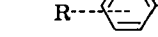


I

MATERIALS AND METHODS

Synthesis of Aryloxyacetonitrile—A mixture of 0.10 mole of the appropriate phenol, 0.10 mole of anhydrous K_2CO_3, 0.11 mole of chloroacetonitrile, and 100 ml. of dimethyl sulfoxide was stirred at 75° for 3 hr. The reaction mixture was poured into 100 g. of ice and the product extracted with methylene chloride. The product was then isolated by vacuum distillation through a 40-cm. Vigreux column. The identification was made with a Varian A-60 nuclear magnetic resonance spectrometer with a 10% $CDCl_3$ solution. The physical properties and yields are listed in Table I.

TABLE I—ARYLOXYACETONITRILE



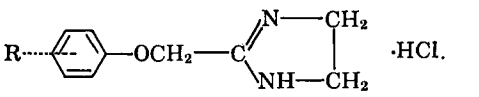
R	Yield, %	B.p., 2 mm., °C.	M.p., °C.[a]
2,6-di Cl	66	114–118	39–40[b]
2,6-di Br	85	135–138	63–64
2-Br, 6-Cl	88	126–128	
2,6-di CH_3	60	125–127[c]	
2,6-di OCH_3	69	133–137[d]	
2,6-di Cl, 3-CH_3	72	138–141	48–49

[a] Thomas-Hoover capillary m.p. apparatus, uncorr. [b] Campbell (15) reports m.p. 40–43°. [c] Barron *et al.* (16) reports b.p. 88–92° (*p*, 5–1.0 mm.). [d] Clark *et al.* (17) report m.p. 94–95° by a different method of synthesis. The authors' material showed no sign of crystallization even at 0°. The NMR spectrum of this material confirmed unambiguously the structure assigned.

Synthesis of the Imidazolines—A mixture of 0.10 mole of the aryloxyacetonitrile, 0.10 mole of 1,2-ethylenediamine monotosylate, and 100 ml. of *o*-dichlorobenzene was heated with stirring under a nitrogen blanket until ammonia evolution ceased. This usually required 1–2 hr. at 170–180°. The reaction mixture was poured into water, one equivalent of NaOH added, and the imidazoline extracted into methylene chloride. After the solvent was removed under vacuum, the residue was dissolved in isopropyl alcohol and a slight excess of anhydrous HCl in isopropyl alcohol added. The resulting imidazoline hydrochloride was then collected by filtration and recrystallized from isopropyl alcohol. Identification was by chloride equivalent and NMR. The spectra in D_2O in all cases were consistent with the expected substitutions in the aromatic regions, as well as the methylene bridge and the ethylene portion of the imidazoline ring. The properties of the imidazolines studied in this investigation are shown in Table II.

Pharmacology—Adult male mice (Cox) weighing

TABLE II—2-(ARYLOXYMETHYL)-2-IMIDAZOLINES



R	Yield, %	M.p. °C. (dec.)[a]	Chloride, Calcd.	Equivalent, Found
2,6-di Cl	57	221–222	281.58	283
2,6-di Br	57	230–231	370.50	365
2-Br, 6-Cl	40	214–216	326.05	329
2,6-di CH_3	76	224–226[b]	240.72	242
2,6-di OCH_3	54	188–190	272.74	272
2,6-di Cl, 3-CH_3	46	229–230	296.61	295

[a] Thomas-Hoover capillary m.p. apparatus, uncorr. [b] Laboratories Dausse, S. A., French pat. 1,312,410 (1962), report m.p. 219°.

18–22 g. were used in these tests. Relative potency was measured by the ability of these compounds to antagonize the HCl-induced writhing response. Writhing was induced by the intraperitoneal injection of 10 ml./kg. of a 0.1 *N* HCl solution. The compounds to be tested were administered orally 30 min. prior to the HCl. The results are expressed as the dose that will effectively prevent 50% of the animals from exhibiting the characteristic writhing. This test has been used as a measure of a drug's ability to cause analgesia (13). The ED_{50} values are given in Table II.

Reactivity Index—Calculated Value—The simple MO calculations were carried out as described by the Pullmans (9). There are many indexes that may be deduced from this type of procedure. The one that appeared to have the most significance for measuring the biological activity of the imidazolines was the energy of the highest occupied molecular orbital (HOMO). This index is a relative measure of the ability of an electron to be transferred to an acceptor molecule.

The calculations were performed on the substituted phenol present in the imidazoline structure. This simplification was made since it could be assumed that any perturbation caused by the imidazole would be insulated from the rest of the molecule by the methylene group. The parameters used for the heteroatoms in this study are listed in previous publications (6, 9).

Experimental Value—The oxidation potentials of the phenols, corresponding to the imidazoline derivatives, were determined with a Leeds and Northrup Electron chromograph type E instrument. This was equipped with 0.6 cm. (1/4 in.) graphite (ultrapure spectroscopic electrodes from Ultra Carbon Corp.) and a calomel reference electrode. The electrolyte was a 1 *M* acetic acid and 1 *M* sodium acetate buffer. The procedure was as follows: the phenol was made 0.001 *M* in methanol, 5 ml. was diluted to 10 ml. with the electrolyte, the solution was placed in the cell, and the potential was scanned from 0 to 1 v. The oxidation potential was recorded as the midpoint of the resulting curve.

Partition Coefficient—The substituent constant π was calculated from the values given by Hansch (5).

Calculations—The MO calculations and the regression analyses were performed by the Computation Laboratory, The Dow Chemical Company, using a Burroughs B5500 computer.

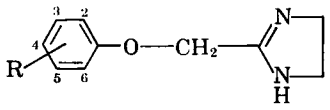
RESULTS

The data collected for this series are shown in Table III. In order to ensure that the calculated value for the energy of the highest occupied molecular orbital was relevant, the oxidation potential was determined for the various phenols. The correlation between the experimental and the calculated values are shown in Fig. 1.

Using the Hansch procedure (1) the biological data in Table III were regressed against the two parameters, π and HOMO. The analysis generated Eqs. 3–5 where

$$\log ED_{50} = -0.445\pi + 1.015 \qquad r = 0.640 \quad r^2 = 0.406 \quad \text{(Eq. 3)}$$

TABLE III—BIOLOGICAL AND CHEMICAL PARAMETERS FOR A SERIES OF IMIDAZOLINE DERIVATIVES



Substituent (R)	ED_{50}[a]	π[b]	HOMO[c]	Oxidation[d] Potential, v.
2,6-diCH_3	0.6	1.36	0.716	0.469
2-Br, 6-Cl	2.0	1.34	0.802	0.642
2,6-di Br	3.2	1.50	0.797	0.634
2,6-di Cl	3.3	1.18	0.808	0.659
2,6-diCl, 3-CH_3	5.4[e]	1.69	0.789	0.625
2,6-di OCH_3	25	−0.66	0.618	0.363

[a] Oral dose in mg./kg. (HCl induced rat writhing test for analgesics). [b] Determined from Hansch's values (5). [c] Energy of the highest occupied molecular orbital of the substituted phenol. The smaller the value, the greater the ease of electron donation. [d] Oxidation potential of substituted phenol determined by means of a Leeds and Northrup Electro chromograph type E. [e] Estimated value derived from the value for a subcutaneous dose of this derivative compared to a similar type of injection of the other derivatives.

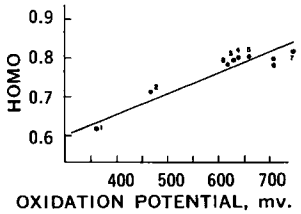


Fig. 1—Plot of HOMO against the oxidation potential (mv.) of a series of phenols. Key: 1, 2,6-di OCH_3; 2, 2,6-di CH_3; 3, 2,6-di Br; 4, 2,-Br, 6-Cl; 5, 2,6-di Cl; 6, phenol; 7, 3,4-di Cl; 8, 2,6-di Cl, 3-CH_3. The values of HOMO and the oxidation potential in mv. for the two phenols not listed in Table III are: phenol (0.711 and 705 mv.), 3,4-diCl (0-811 and 765 mv.).

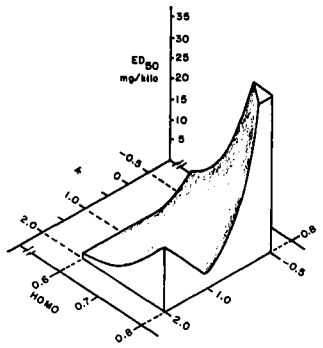


Fig. 2—Structure-activity surface generated by Eq. 5. The most potent analgesic has the smallest ED_{50}. The data for this figure are listed in Table III.

$$\log ED_{50} = +0.655\pi^2 - 1.00\pi + 0.455 \qquad 0.728 \quad 0.531 \quad \text{(Eq. 4)}$$

$$\log ED_{50} = 0.945\pi^2 - 1.850\pi + 7.90\,(\text{HOMO}) - 5.117 \qquad 0.975 \quad 0.944 \quad \text{(Eq. 5)}$$

r is the correlation coefficient. The good correlation

shown by r is indicated by r^2 (*i.e.*, 94.4% of the variance is "explained" by Eq. 5). An F test indicated that the inclusion of the HOMO term in Eq. 5 is significant at the 5% level, whereas Eqs. 3 and 4 were considerably less than 95% significant. There was no correlation when the biological response was regressed using the HOMO parameter by itself (Fig. 2).

DISCUSSION

One of the important items to emerge from this study is the role played by the substituent constant π. As Eq. 4 indicates 50% of the activity may be correlated with π. Consequently, the balance between the hydrophobicity and hydrophilicity of a chemical (as expressed by π) would seem to be critical. One interpretation of these data is that the partition coefficient has a strong influence on the actual amount of drug found on the receptor site. This occurs through a "random walk" process in the organism and finally association with the site itself. The recent work of Hansch (4) dealing with the influence of π on the binding of small molecules to bovine serum albumin helps to substantiate this hypothesis. As the drug builds up a concentration on the receptor site the intrinsic reactivity takes over and through the formation of a charge-transfer complex elicits the observed response. Green and Malrieu (11) made use of the concept of charge-transfer complex in study of a series of indole derivatives. In essence this type of complex may be formed when a compound capable of donating electrons is mixed with a receptor possessing the capacity to accept electrons.

In a recent review article on the molecular basis of drug action, Mautner (14) raised the question as to the molecular significance of the Hansch-type of correlation. The authors agree that the correlation does not predict a specific mechanism where the reactivity of the actual functional groups is implicated. The phosphorylation of the esteratic site in cholinesterase, as a mechanism for inhibition, is an example of a molecular description (6, 7). On the other hand, these correlations do provide a working model for speculating on the mode of action. In the present case, the interpretation of the results suggests a model which can be tested by further experimentation. The model that emerges consists of a receptor site which has the ability to accept electrons from an electron-donating drug. The association is probably similar to a Michaelis-Menten-type situation, hence the biological activity would have a short half-life relative to a drug that forms an actual C—C covalent linkage. The ability of the two parameters, π and HOMO, to correlate with the biological response would also indicate a type of drug that was not very selective in its action. While this model does not give a molecular mechanism, it may provide insight as to how this particular series of chemicals elicit their analgesic-like activity.

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Keyphrases

Imidazolines—synthesis
NMR spectrometry—identity
Pharmacological activity—imidazolines
Structure-activity relations—analgesic
Reactivity index—molecular orbital technique