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On the Structure of Medicinal Chemistry

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The great advances in biochemistry and molecular biology, the development of physical organic chemistry, and the availability of large computers are creating opportunities for restructuring medicinal chemistry. The enormous volume of scientific results relevant to medicinal chemistry which appear with each new round of the journals forces us to make greater efforts to bring the information together in more meaningful patterns. The QSAR paradigm redirects our thinking about structuring medicinal chemistry.

It is a signal honor and at the same time embarrassing to be the first recipient of the Smissman–Bristol Laboratory Award in Medicinal Chemistry. So many people have helped me in the development of a quantitative multiple-parameter approach to structure–activity relationships (QSAR) that I feel uneasy in accepting the award for our effort.

Pasteur said that chance favors the prepared mind. The preparation of my mind occurred during a most enjoyable and profitable 15-year cooperative study of plant-growth regulators with Robert Muir, Professor of Plant Physiology at the University of Iowa. Muir's careful and patient testing of scores of phenoxyacetic acids against uncounted thousands of oat sections gradually made it evident to us that hydrophobic as well as electronic and steric characteristics of the substituents of a parent molecule were crucial in determining activity.[1] Moreover, it was clearly not possible to hold all variables constant and study a single variable at a time. With P. P. Maloney we found that partition coefficients from the octanol–water system, first studied by Collander, appeared to serve as operational descriptors of hydrophobicity.[2] At this point in our work Professor Toshio Fujita from Kyoto University joined our effort and made the suggestion that we follow the lead of Robert Taft and make a linear combination of hydrophobic and electronic constants to define these two different substituent properties. Professor Fujita then spent the better part of 2 years studying octanol–water partition coefficients (P) to establish the additive–constitutive character[3] of log P which had only been hinted at by Collander.

Development of our quantitative structure–activity technique (QSAR) has depended completely on the use of the computer. Our work would not have been possible without the patient instruction in its use by Donald McIntyre, Professor of Geology at Pomona College. My shortcomings in the critical area of physical chemistry were mitigated by many discussions with my colleague, Nelson Smith, Professor of Physical Chemistry at Pomona.

The organization of the massive amount of data, its storage, and publication have, even with the help of the computer, taxed us sorely. Since 1962, Mrs. Billie Blaney has provided vital help in this area.

In addition to the crucial help mentioned above, I have been aided by so many students and colleagues that it is not possible to mention all of their names.

It is instructive to consider the development of the QSAR paradigm[4] in a wider sense. One might pick its birthdate as about 1870 when Crum-Brown and Fraser[5] advanced the idea that

$$\text{biological response} = f(C) \qquad (1)$$

By this they meant that biological response (BR) was a function of chemical structure (C). They stated that it should be possible to develop a calculus of SAR by making small changes in chemical structure and relating these to BR. The real barrier to carrying out their dream was that of defining the significant parameters of chemical structure in numerical terms.

An important part of this job was completed in principle at the turn of the century when Meyer and Overton used oil–water partition coefficients as a hydrophobic scale to rationalize the narcotic action of simple organic molecules. They did not go so far as to formulate an equation for the relationship. This effort stalled because it was not yet clear how to take into account the steric and electronic properties of compounds.

A third of a century of qualitative study of the electronic effects of substituents initiated by the "English School" was culminated by Hammett's formulation of numerical constants (σ) for the electronic effects of substituents.[6] Hammett's simple, but extremely important, idea was brilliantly developed by Taft who defined aliphatic σ constants and steric constants (E_s)[7] and then showed how σ could be factored into inductive and resonance components.

The availability of computers in the early 1960's was the key piece for the general development of the QSAR paradigm of eq 1. It simply remained for our group at Pomona to demonstrate that some (but by no means all) of the confusion and frustration of building structure–activity relationships in biomedicinal chemistry could be cleared up via substituent constants and regression analysis. This has been done by rewriting eq 1 as

$$\Delta BR = f(\Delta electronic + \Delta steric + \Delta hydrophobic + \Delta polarizability) \quad (2)$$

The terms on the right of eq 2 refer to changes produced in a parent drug by addition of substituents. Constants such as π, σ, E_s, and MR from model systems can be used to seek meaningful solutions for eq 2. We now have about 2000 biomedical QSAR on about 20000 organic compounds in our data bank. For comparative purposes, we have about 2000 QSAR on 20000 compounds from simple organic reactions.

Of course there are still problems. One of the best stories about these is by someone who said, "Hansch's approach reminds me of the way they used to weigh sheep out in the back country in Australia. No scales were available so a rail was placed across a rail fence with a sheep on one side and rocks piled on the other. When the two were balanced, one guessed at the weight of the rocks and of course from this one knew the weight of the sheep." Our problem is more than the matter of weights; we are not yet sure of the shape of the "rail" connecting biological response with our "weights"!

The QSAR paradigm is beginning to more strongly determine the course our work takes. For example, the seriousness of the problem of collinearity has not been appreciated by medicinal chemists and, in fact, little can be done about it until one has numerical tools with which to work. The problem is trickier than intuition would lead one to expect.

Consider the following two sets of substituents one might select

I		II	
CH_3	NHC_6H_5	CH_3	CH_2CH_3
NO_2	$OCH_2CH_2CH_3$	CF_3	$NHCOCH_3$
$COCH_3$	$SO_2CH_2CH_2CH_3$	F	$CONH_2$
$C{\equiv}CH$	I	CN	SO_2NH_2
SCH_3	CH_2Cl	NO_2	OCF_3

to study the interaction of a parent drug with an enzyme. Is either suitable for our purpose? That is, do the variables π, $\mathcal{F}$, $\mathcal{R}$, and MR for the hydrophobic, inductive, resonance, and polarizability for either set behave independently? It is impossible to make such a decision by inspection of the data, even with tables of constants. However, the degree of independence is easily seen from the squared correlation matrix

I	π	$\mathcal{R}$	$\mathcal{F}$	MR
π	1.00	0.94	0.30	0.32
$\mathcal{R}$		1.00	0.32	0.38
$\mathcal{F}$			1.00	0.09
MR				1.00

II	π	$\mathcal{R}$	$\mathcal{F}$	MR
π	1.00	0.09	0.08	0.26
$\mathcal{R}$		1.00	0.15	0.03
$\mathcal{F}$			1.00	0.03
MR				1.00

It is obvious from matrix I for set I that the vectors π and $\mathcal{R}$ are almost perfectly collinear ($r^2 = 0.94$ where r is the correlation coefficient for the linear relationship between π and $\mathcal{R}$). There is only one example of clear independence between vectors in data set I and that is $\mathcal{F}$ and MR. Matrix II shows that there is some collinearity between π and MR but that all other combinations are independent. If we made and tested the ten derivatives of set I and if either hydrophobic bonding or resonance were important, we would be in the ambivalent position of not knowing which was the important variable or if some combination of the two was responsible for changes in activity. Our experience, as well as that of others, is that the majority of published structure–activity studies suffer from poor experimental design because the collinearity problem is present to a serious degree.

Matrices such as I and II should always be constructed (insofar as constants are available) throughout a research project, even when one has no intention of eventually formulating a QSAR. Such a practice gives one better perspective in the design of molecular probes.

The "systematic" modification of organic compounds to develop better drugs and pesticides has been a multihundred million dollar business of many years; yet discussion of the problem and how one should go about the efficient selection of substituents is not to be found in the pre-QSAR literature. The new paradigm is forcing us to seriously consider proper strategy. Leaving aside variables which are not yet readily parameterized, such as highly stereospecific interactions, metabolism, etc., there are still difficulties in designing a "best" set of derivatives. From the literally billions of derivatives which can be made from a molecule as simple as benzoic acid, what constitutes a truly representative sample? Confining our view to chemical changes which can be parameterized, there are three major problems in modifying an interesting "find": (1) minimization of collinearity; (2) maximization of variance; (3) the mapping of substituent space with the fewest number of probes.

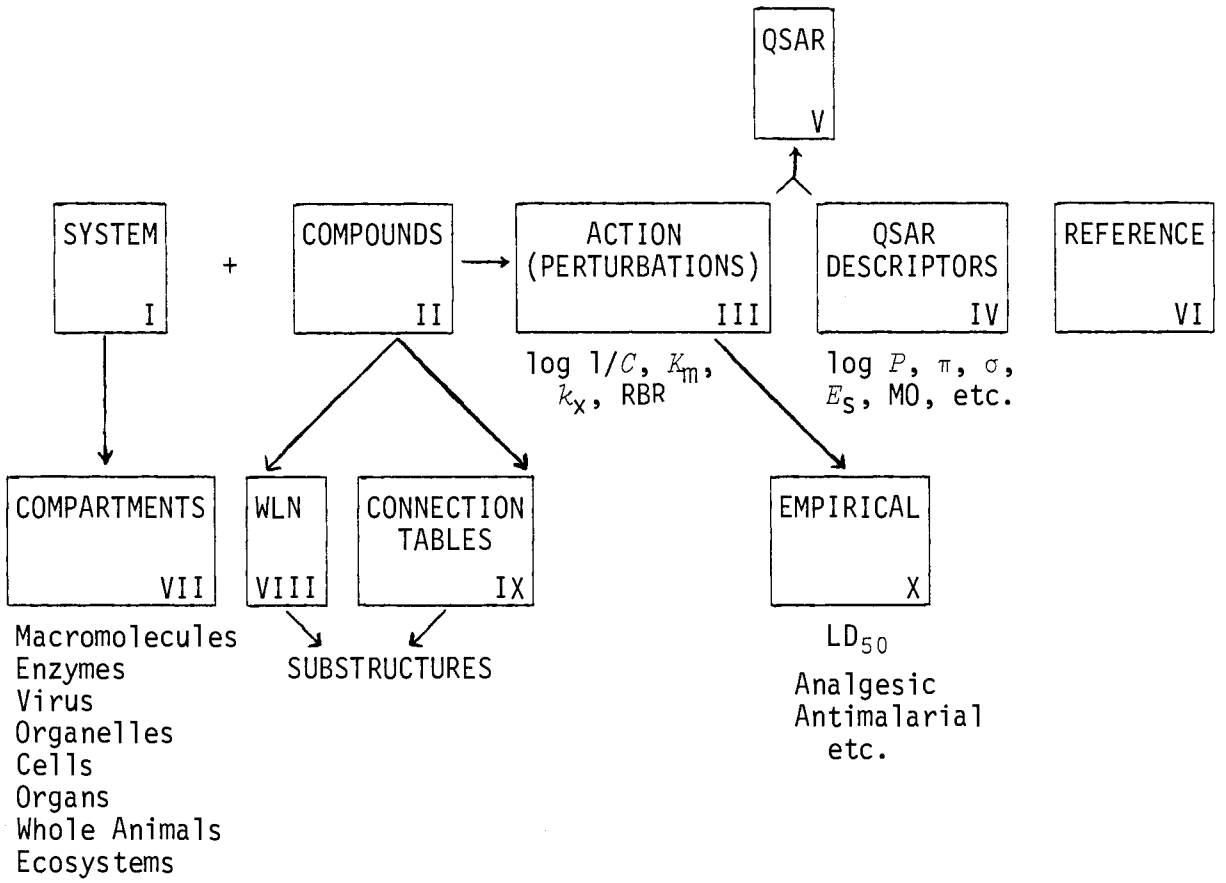
Starting with a paper by Craig[8] in which the advantages of making plots of π vs. σ (often called a Craig plot) were considered, the discussion has become more varied and general. The Topliss[9-11] decision tree and Craig-type plots, although less rigorous, provide machine-independent techniques for the derivatization of a parent structure. The use of cluster analysis has been discussed as a means for minimizing collinearity in multidimensional space.[12] Most recently, an interesting algorithm has been devised by a Burroughs–Wellcome group[13] for the systematic exploration of substituent space. While none of these techniques solve all of the problems in drug modification, they do enable us to commence a more organized effort. Strategy in drug modification is becoming a subject in its own right.[11b]

As our understanding of drug action at the molecular level advances through the development of biochemistry and molecular biology, it becomes more urgent to organize and systematize the interactions of organic compounds with as many biochemical systems as possible. Those areas in which enzymology is advanced have greatly aided the development of drugs such as allopurinol and trimethoprim[14] by Hitchings' group. The outstanding studies by Umezawa,[15] Baker,[16] and others[17] show that more rational approaches to drug development are emerging. Still, there is a crying need for better methods of organizing our growing pool of information.

Following our QSAR paradigm has resulted in the computerized system of data management of Scheme I. Except for connection tables (compartment IX), Scheme I is now in operation. Although we have made some simple studies with it,[18] our main effort is still directed toward construction of a large, well-balanced data base. The basic thought behind Scheme I is that a well-defined system (be it an enzyme or the state of New Jersey) interacts with a set of congeners to yield a set of perturbations (III). From these results (where possible), one formulates a correlation equation (V) via physicochemical constants (IV). With the enormous amount of old data in the literature and the rapid outpouring of new data, one soon begins to accrete information on many hundreds of different types of systems (VII) interacting with tens of thousands of organic compounds. The proper naming of these systems is a serious problem in data management.[19]

As our data base developed, the inadequacy of the nomenclature of organic chemistry forced us to use Wiswesser Line Notation (WLN VIII). By means of WLN

Scheme I



such questions as, "What systems are perturbed by $-SO_2NH_2$?" can be studied. Eventually, WLN will be combined with Connection Tables similar to that of the Cross-Bow system.[20]

The quantitative measure of potency in our system (degree of perturbation) is normally defined as log $1/C$ (where C is the molar concentration of drug producing a standard response), a rate or equilibrium constant, or a relative biological response (usually compared to a standard drug). The more difficult aspect of action is its qualitative definition. This is now entered (X) as hypnotic, antiinflammatory, antibacterial, etc. While this will long continue to be the only way to classify many drugs, it is becoming possible to also label the drug in accord with its action at the molecular level, e.g., dihydrofolate reductase, carbonic anhydrase, or ATPase inhibitor. In the long run it would seem that we must categorize organic compounds with respect to the type of macromolecules or constellations of macromolecules by means of which they produce their effect.

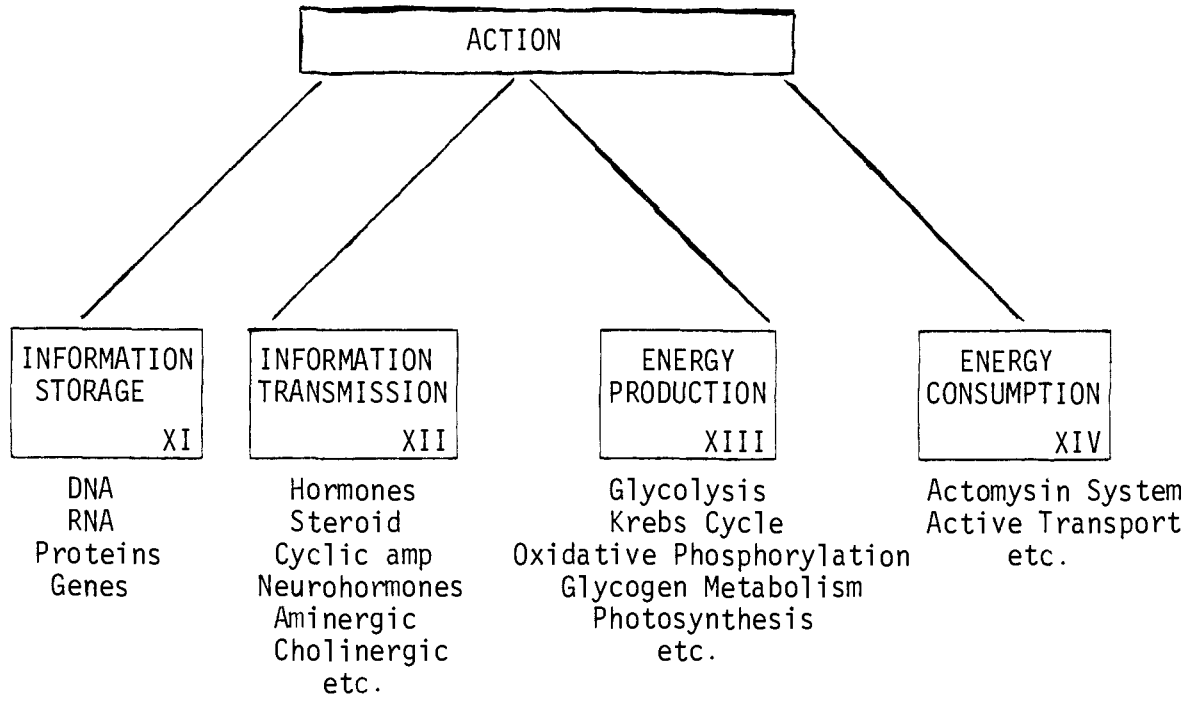
Our knowledge in this area must be built up systematically so that we shall know at the outset of a research project which types of functions to avoid as well as on which we should concentrate. In principle, one would like to know how all organic compounds react with all macromolecular systems; such information is forever beyond our reach. What we can do is make intelligent guesses on the basis of organized experience as to how a given compound might react with enzymes or other macromolecules. The problem is, how should medicinal chemistry be structured to aid such guessing?

We can make inferences from studies with model systems if they can be organized effectively. For example, it is now understood that the inhibitor of one hydrolase will likely inhibit other hydrolases. Baker showed that the four dehydrogenases, malate, glutamate, lactate, and glyceraldehyde 3-phosphate were all inhibited by a set of quinoline-3-carboxylates. The formulation of the four appropriate QSAR shows both some of the similarities and differences in the way the four dehydrogenases react to a set of over 70 inhibitors.[21] This raises a serious problem which must be guarded against in attempting to develop new drugs by studying their interaction with a single enzyme. Indeed, there are so many macromolecules in an animal with which a prospective drug might react that all of the necessary in vitro studies could never be made. A mouse will probably forever remain the cheapest instrument for gathering information on the incredibly complex interactions of an organic compound with the macromolecules of a living system. However, sticking congeners in a mouse is the hard way to discover critical differences between isozymes or isoreceptors; one gets a fully integrated response from the mouse and has little idea where to charge the difference in activity of two congeners.

The great difficulty in finding new drugs has been analyzed by Spinks[22] who estimates that one new drug arises out of each 200000 new compounds. He suggests that one can expect to find an anticancer drug out of each 400000000 randomly tested compounds. This is about 100 times the number of known organic compounds. One might question this figure since there are about 40 drugs now in clinical use against cancer; however, none of these is truly effective in the sense that antibiotics are. Spinks' estimate of 4×10^8 randomly tested compounds for each effective antitumor drug may not be as wild as it seems at first glance.

An important part of our system which needs much more attention is the Action compartment (X). We have begun to speculate about factoring this field, possibly along the lines of Scheme II. Of course, in the last analysis, all of the compartments of Scheme II are interrelated so that it is not clear at present how the coding should be de-

Scheme II



limited. It is easy to see that a set of congeners inhibiting cholinesterase could be coded as hydrolase inhibitors interacting with neurohormones; however, there is almost no end of the connections one could attempt to make.

There are, naturally, many obvious and simple ways in which computerized SAR can be used.[18,19,23] Lists can be generated so that one can quickly see if a given article has been analyzed; all of the QSAR on hydrolases or a single enzyme such as hexokinase can be listed; a summary of which substituents are not well behaved or what kind of log P_0 (ideal lipophilicity) has been identified,[19] etc., etc. While the system can handle single molecules and make nonparametric analyses, we are still limiting our studies to sets of congeners which can be cast into a reasonably good QSAR. In what ways can we use QSAR to establish some elements of medicinal chemistry?

As a first step, we can begin to group together like kinds of phenomena. For about 10 years we have been collecting all kinds of QSAR from the literature as we run across them. The kinds and variety are so great that there is no clear picture of what is accruing. We have paused three times in this process to find out what was being collected with respect to the simplest cases of hydrophobic interactions. Three papers were written from the following three queries of our data base: (1) print out all equations of the type, $\log 1/C = a \log P + b$;[24] (2) print out equations of the above type where $\log 1/C$ is not the dependent variable;[25] (3) list all equations of the type, $\log 1/C = a \log P + b (\log P)^2 + c$.[26]

The first query yielded 138 examples which appear to fall roughly into three groups. Fifty-seven have slopes of 1.01 ± 0.13, 71 have slopes of 0.66 ± 0.12, and 10 have slopes of less than 0.4. The high slopes were found in systems where red blood cells, nerve cells, or yeast cells were perturbed by sets of congeners. The intermediate slopes are associated with protein or bacterial perturbations. Nothing can be concluded from the small set with slopes less than 0.4. We learned from these studies that the slopes were dependent on the system and not on the type of congener. It now appears that almost any biochemical system (mouse or enzyme) reacts in a similar way to a set of nonspecific lipophilic probes. This is a generalization of the Meyer–Overton theory to the molecular level.

Living systems abhor foreign lipophilic compounds because macromolecules in the important biochemical process use lipophilic pockets to trap and position their substrates as well as to position themselves in the proper place in their own constellation. As Brodie suggested long ago, the liver microsomes have in part evolved for the purpose of destroying unwanted lipophilic molecules.

From these simple equations, where specificity is not high, one can make reasonable and, I believe, reliable estimates of the activity of hundreds of thousands of simple organic compounds in thousands of different systems. This may not excite a chemist charged with the awful task of finding a profitable new drug but, as such equations are formulated for systems of higher and higher specificity, they will produce structure in the field of medicinal chemistry.

While medicinal chemists have long been aware that one cannot go on forever making a parent structure more lipophilic to get more activity, it was not clear until the development of QSAR that one could expect[27,28] and find (often but not always) a rather clear "parabolic" relationship between lipophilic character and biological response in a set of congeners. It has now been established for a few systems such as hypnotics,[29] antibacterials,[30] and antitumor drugs[31] that sets of congeners, presumably acting on the same site by the same mechanism, have the same log P_0 (ideal lipophilic character). Query no. 3 yielded 230 equations for which log P_0 could be defined reasonably accurately. When we have a good selection of log P_0 values for many types of compounds in a good selection of systems, these constants will be of enormous help in moving from in vitro enzyme studies to in vivo tests.

Bioisosterism has been one of a few guiding principles in drug research. The QSAR paradigm leads us to a deeper understanding of this principle. If we define bioisosterism phenomenologically by saying that when two molecules give the same biological response in a standard system, they are bioisosteric,[23] then two types of bioisosters are possible. True bioisosters would yield identical (quali-

tatively and quantitatively) biological responses; partial bioisosters would yield qualitatively the same but quantitatively different responses. This definition can be explored in terms of QSAR.[12] If two congeners are true bioisosters (identical responses), the occurrence can be explained in two ways. Assume that activity is determined in terms of π, σ, and E_s of the substituents. It is possible to find two substituents which have almost the same values for these three constants; e.g.

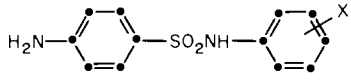
	σ	π	E_s
Cl	0.23	0.71	0.27
Br	0.23	0.86	0.08

Compounds with substituents whose constants are very close in value yielding the same biological response can be called isometric bioisosters.[12] They are more apt to be found when only one or two of the above parameters are significant. For example, in benzoates causing 20-mV increase in mollusk membrane potential, we find the following bioisosters.[23]

	log $1/C$	log P
2-[2,3-$(CH_3)_2$-C_6H_3NH]$C_6H_4COO^-$	3.48	0.28
2-OH, 3,5-Cl_2-$C_6H_2COO^-$	3.48	0.23

Viewed in terms of traditional symbolism of organic chemistry, the two derivatives seem grossly different; to the nerve membrane seeing the compounds only in terms of lipophilic character, they are identical. The data set of 30 benzoates from which these congeners come is highly correlated (r = 0.979) with an equation linear in log P.

In a group of *Escherichia coli* inhibiting sulfonamides of the type



the following congeners are bioisosteric.[32]

	log $1/C$	σ	π
4-Cl	4.80	0.23	0.70
3-OC_2H_5	4.88	0.12	0.62

Similarity in substituent constants = similarity in activity.

The above examples represent isometric bioisosterism. The following pair of congeners complexing with alcohol dehydrogenase and DPNH illustrate nonisometric bioisosterism.

X-$C_6H_4CONH_2$

	log $1/K$ obsd	log $1/K$ calcd	σ	π	E_s
4-F	−2.6	−2.5	0.06	0.27	0.78
4-NO_2	−2.6	−2.6	0.78	0.18	−1.28

The QSAR for this congeneric set is[33]

$$\log 1/K = 0.45(\pi\text{-}4) - 0.80\sigma - 0.23(E_s\text{-}4) - 2.37$$

n	r	s
14	0.953	0.168

In this example, π and E_s apply only to 4-substituents while σ applies to both 3- and 4-substituents. This illustrates the highly directional nature of hydrophobic and steric interactions. Electron-releasing groups promote binding and large groups in the 4 position also favor binding, possibly by causing a conformational change (negative coefficient with E_s-4). The unfavorable electronic effect of 4-NO_2 is offset by its large steric effect and for 4-F its more favorable electronic effect is offset by its lack of steric effect. Nonisometric bioisosterism results when two or more properties of a structure change in different ways but combine additively to produce the same degree of biological activity.

A survey of our present bank of QSAR finds that there are more equations correlating the interactions of ligands with enzymes than any other kind. Good correlation equations for sets of congeners with enzymes are the rule rather than the exception. This presages a most important role of QSAR in drug development. The enormous burst of activity in the study of isozymes[34] shows that one normally expects to find multiple structures of almost all enzymes. Advantage can be taken of these differences in the design of inhibitors with great specificity for enzyme of the pathogen rather than that of the host.[35] Before long we shall have structures from x-ray crystallography of isozymes from host and pathogen. Correlation analysis will be a most important tool in maximizing the fit of ligands to one isozyme and minimizing the fit to the other isozymes. While one can spot hydrophobic and polar pockets from x-ray-determined structures, one cannot estimate with much precision the size of the groups which will fit into such pockets nor, more importantly, the strength of such interactions. Such information will have to be gleaned from a study of well-designed molecular probes. QSAR will play an important part in developing selective toxicity at the enzyme level. Parallel QSAR studies with animals on metabolism, toxicity, and efficacy will ultimately help in the difficult compromise necessary in selecting a final molecule for the clinic.

It is unlikely that Crum-Brown and Fraser foresaw that an enormous drug and pesticide industry would arise within 100 years and that the solution of eq 1 (via solar plexis or computer) would become the lifetime occupation of tens of thousands spending billions of dollars. We are just at the beginning. Man's striving for better health, longer life, and an understanding of his interactions with the sea of chemicals through which he moves provides a huge pressure for obtaining better solutions for eq 1.

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Synthesis and Adrenoreceptor Blocking Action of Aziridinium Ions Derived from Phenoxybenzamine and Dibenamine†

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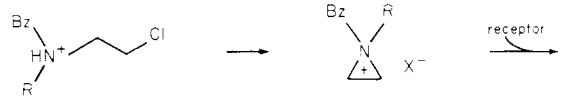
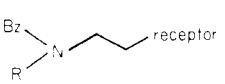
Crystalline perchlorate salts of aziridinium ions derived from phenoxybenzamine and dibenamine were prepared. Both aziridinium ions were tested on the rat vas deferens and found to possess α-adrenergic potencies which were nearly identical with those of the parent compounds. The hydrolysis rates of phenoxybenzamine and dibenamine aziridinium ions (**2a,b**) in physiological medium were found to be 6.04×10^{-4} and 8.35×10^{-4} sec^{-1}, respectively. The rates of cyclization of the parent amines to **2a** and **2b** in aqueous medium were 1.9×10^{-2} and 7.2×10^{-3} sec^{-1}, respectively. The potencies and kinetic profiles indicate that the aziridinium ion is the only active species in α-adrenergic blockade. Moreover, differences in potency between phenoxybenzamine and dibenamine appear to be exclusively to a difference in receptor affinity rather than to a difference in intrinsic alkylating ability.

Phenoxybenzamine (**1a**) and dibenamine (**1b**) are employed extensively as tools in pharmacologic studies. Although it has been generally accepted that the nonequilibrium blockade of α-adrenergic receptors by these drugs is mediated through the corresponding aziridinium species (**2a,b**), no unequivocal evidence has been reported which establishes the intermediacy of this reactive intermediate.[1,2] The acceptance of the mechanism for receptor inactivation is based principally on an extensive collection of indirect evidence compiled since Nickerson and Goodman[3] first proposed the formation of an aziridinium ion as a prerequisite for covalent bond formation with the receptor.



1a, R = CH_3CHCH_2OPh **2a**, R = CH_3CHCH_2OPh
b, R = Bz **b**, R = Bz



Historically, the approach taken for the study of the activities of ions related to **2** has been by the use of solutions containing predicted concentrations of both parent amine and the intermediate.[4-6] The levels of intermediate were estimated by sequential titrimetric determinations of hydrogen ion and chloride ion concentrations. Since the time frames of such methods might not be sensitive enough to accurately measure the rapid dynamic processes taking place in these systems, a more definitive study of the kinetic and pharmacologic behavior of 1 and **2** would be instructive.

The most apparent manner in which to test the activities of **2** is to quantitatively convert 1 into **2**. Unfortunately, the isolation and testing of an aziridinium ion intermediate for pharmacologic activity have been accomplished in only one previous case. Allen and Chapman[7] and Graham[8] found that the in vivo activities of ions derived from several *N*-ethyl-*N*-chlorobenzyl-2-chloroethylamines (as the picrylsulfonate salts) were roughly parallel to their concentration. To our knowledge, no detailed investigation has been published on the isolated aziridinium ion (**2**) derived from phenoxybenzamine or dibenamine. Because of their extensive use in pharmacology, we have undertaken a study which includes the synthesis and characterization of **2a** and **2b**, their rates of formation and decomposition under physiological conditions, and their in vitro activities.

Chemistry. The aziridinium compounds **2a** and **2b** were synthesized and isolated as the stable perchlorate salts (X = ClO_4^-) by the use of $AgClO_4$, according to the method of Leonard and Paukstelis.[9] Thus, the free base of racemic **1a** was treated with excess anhydrous $AgClO_4$ in acetone resulting in the formation of **2a** and AgCl. As expected,[10] the aziridinium salt underwent decomposition when stored for extended periods in acetone solution but was stable in, and recrystallizable from, CH_2Cl_2. Similarly, treatment of the free base of **1b** with anhydrous $AgClO_4$ in acetone afforded salt **2b**, which was also recrystallizable from CH_2Cl_2.

A careful proof of structure for **2a** and **2b** was undertaken, to eliminate the possibility of the piperazinium dimer **3**, although such compounds have been shown to be

† This paper is dedicated to the memory of Edward E. Smissman.