META. 1. A Program for the Evaluation of Metabolic Transformation of Chemicals

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A new metabolism program, META, is introduced. In this paper, the basic principles on which the program operates are described. META is an expert system, capable of predicting the sites of potential enzymatic attack and the nature of the chemicals formed by such metabolic transformations. It operates from dictionaries of transformation operators, created by experts to represent known metabolic paths.

INTRODUCTION

Pathways of intermediary metabolism of endogenous physiological chemicals were often discovered through concentrated efforts involving the isolation and purification of individual enzymes and identification of precursor−product relationships that define consecutive enzymatic reactions. This process has continued, albeit at a slower pace, as emphasis in the biological sciences has shifted toward unraveling regulatory phenomena. The efforts to discover endogenous intermediary metabolites and their mechanisms of formation have been motivated by fundamental curiosity regarding the function of the human body as well as the desire to discover the basis for disease processes and potential approaches for intervention and cure.

With the burgeoning use of chemicals for the coatings industry, cosmetics, food additives, control of pests in agriculture, and chemotherapy of humans and animals, there has been an exponential increase in the numbers and avenues of exposure of people, animals, and crops to exogenous chemicals. In each of these encounters the chemically-induced effects on the organism result from the composite effects of the parent agent and its metabolites. Accordingly, there has been a sustained effort to develop methodologies capable of uncovering the relation between the structure of the chemicals and their activity and of defining the pathways of biotransformation of exogenous chemical agents in order to understand which chemical entities are responsible for the desired as well as the toxic effects of the agents.

In vertebrate animals and humans, the liver is the most abundant source of biotransformation enzymes that evolved presumably in repsonse to the toxic insults of alkaloids in the plants that were used for food. Most xenobiotics are converted concurrently or consecutively to multiple metabolites by hepatic microsomal and cytosolic enzymes. Other tissues, however, including plasma, kidney, lung, and the gastrointestinal tract also contribute to the biotransformation of exogenous agents. In the case of environmental xenobiotics, the routes of exposure often are more likely inhalation or direct contact with the skin, rather than oral ingestion or intravenous injection. In these cases, relatively low levels of enzyme activity in the epithelial cells may contribute significantly to local metabolic toxic activation.

In many cases it has been possible to identify the bioactive forms of specific chemical agents and, based on this knowledge, to predict the metabolic conversion and bioactivity of analogous compounds. In a prospective sense, this approach has led to the development of successful prodrugs (inactive parent drug/therapeutic metabolite), and the avoidance of certain types of drug interactions and toxicity.

As more new chemical agents are designed for the variety of purposes alluded to above, there is an increasing need to predict accurately and completely the nature and bioactivity of their metabolites. Hence, we undertook a study aimed at developing a computer program for predicting the metabolic products of chemical agents and for evaluating the potential desirable bioactivity and/or potential toxicity of these metabolites. It is based on an expert evaluation of their chemical structure and does not need prior knowledge about the actual metabolism of the compound.

There have been a number of other attempts in this direction, but the literature does not offer much background in this area.[1−5] Indeed, while certain pharmaceutical companies may have engaged in such a task (e.g., Upjohn Company), they have not published their results in any substantial detail, nor have they made their programs generally available. To our knowledge, there has been only one commercially available computer program[6] that claims to predict metabolites. However, there are a number of programs developed for the purpose of computer-assisted organic synthesis planning which may employ principles similar to those used in our program.[7−9]

In this paper, we describe the basic operation of the metabolism program. For easy reference in the rest of this paper, we will refer to this program as "META". Subsequent papers will be devoted to the description and the development of the metabolism dictionaries used by META to actually predict the metabolic transformation of chemicals.

PROBLEM ANALYSIS AND METHOD

One of the major objectives in the development of this program was to create an interactive means of predicting possible metabolic transformations of any given compound, irrespective of whether its metabolites are known or not.

A stepwise approach was used to evaluate the stable metabolites according to the sequence in which they are found. This models the fact that experimentally observed metabolites may be the result of several metabolic steps. These biotransformations are coded and compiled in a *dictionary* containing relevant information about the structural constraints governing the specificity of each metabolic transformation. In addition, a dictionary of spontaneous

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reactions is available to detect and process unstable intermediates generated by some of the primary metabolic reactions.

The major enzymes known to be involved in the metabolic transformations of xenobiotics must be identified. Biotransformation rules describing the essential activity of each class are formulated. The program must be able to recognize and apply these rules and display the primary, secondary, tertiary, etc. potential metabolites of a parent compound, conceivably leading to a host of metabolites of decreasing relevance.

In certain unusual circumstances such as predicting the identity of an undetectable trace toxic species, the ability to examine very minor metabolites may be valuable. In most cases, however, it will only be necessary to categorize the metabolites initially according to their relative rates of formation and susceptibility to further metabolism, i.e., their relative abundance and half-life of elimination. This selection will be based on a comprehensive compilation of literature data on relative occurrence of specific metabolic conversions.

Relative Biological Reactivity. The relatively abundant metabolites can be further subdivided according to their potential for biological activity using various criteria, e.g.; (i) therapeutically active, (ii) inactive and excretable, (iii) inactive and retained, and (iv) potentially toxic, etc. Again in this prioritization mode, the system will be alerted to special cases. For example, certain compounds that are conjugated with glutathione and taken up by the kidney may become renal toxins by the action of the β-lyase enzyme, rather than being excreted eventually as mercapturic acid conjugates. Certain metabolites excreted into the intestine via the bile as glucuronide conjugates may be cleaved by β-glucuronidase and the parent compound reabsorbed, i.e., enterohepatic circulation.

Biodisposition of Metabolites and Tissue Distribution of Metabolic Enzymes. Different local relative amounts of various metabolites will arise in different tissues depending upon the route of administration, oil/water partition coefficient, protein binding, and selective uptake mechanisms for the chemical compound in question. Moreover, the relative amounts of cytochrome P-450 isozymes and phase II conjugation enzymes vary from tissue to tissue not only with respect to their constitutive levels but also with respect to their relative inducibility and degree of repression caused by exposure to various chemical agents. Thus a complex decision tree had to be developed to rank the importance of potential metabolites relative to specific bioactivities.

Interspecies and Interindividual Variations in Metabolic Enzymes and Tissue Distribution. The majority of the data in the literature on metabolic pathways and relative abundance of metabolites is for animal species other than humans, and there are numerous examples of differences in relative abundance of primary metabolites, relative activities, and inducibility of enzymes, etc. among the animal species and humans. Thus, the first line survey of potential metabolites will be based upon similarities among the species, but specific applications to individual species will need to be based upon a scrutiny of the differences among the species. In special cases, genetically-based interindividual variations in metabolism should be considered such as the polymorphisms associated with N-acetylation and certain P-450 mediated oxidations.

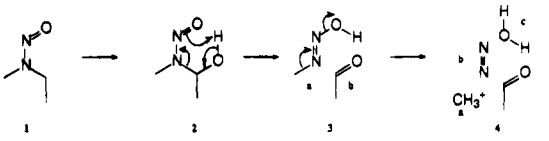

Figure 1. One possible pathway of P-450 hydroxylation of methylethyl-*N*-nitrosamine.

The META Program. The META program has been developed to be consistent with another program developed in our laboratory, the Computer Automated Structure Evaluation (CASE) program.[10] Its objectives are to determine the potential metabolites that could be produced from a chemical entered into the program. The molecular structures can be entered by a variety of methods. Simple molecules can be entered by typing in the chemical formulas. More complex molecules can be entered through their KLN code,[1] SMILES code,[12] graphics, or through a MOL file.

The program operates in conjunction with a variety of dictionaries, each containing information, compiled independently, that list target sites on the substrates and the metabolic transformations that the enzyme will catalyze. Each dictionary represents a particular metabolic model, e.g., individual animal species or different organs.

We define as a *target fragment* a molecular fragment believed to be recognized by a specific enzyme and a *product fragment* as the structure of the target fragment, after it has been metabolized by the enzyme. Each target−product fragment pair is called a transformation operator or simply a *transform*.

The product of a biotransformation is often an unstable intermediate and will react spontaneously. To execute this operation, another dictionary, containing transforms that model spontaneous reactions is consulted. This operation is repeated until the program is satisfied that the resulting molecule is stable in that no transforms in the spontaneous dictionary apply.

Following is an example of a sequence of transformations. Figure 1 illustrates one of the paths followed by the mammalian P-450 metabolism of methylethyl-*N*-nitrosamine, **1**. The CH_2N group of **1** is recognized as a target fragment by a P-450 transform. The product fragment is CH(OH)N. The transform used is

Find:	CH2−N−
Replace with:	CH −N− ⟨1−OH⟩

which is the hydroxylation of an aliphatic carbon α to a nitrogen atom. The molecule resulting from this transform is **2**, $CH_3CH(OH)N(N{=}O)CH_3$.

The spontaneous reactions dictionary will then be consulted, and it will signal that the CH(OH)NN=O moiety is intrinsically unstable and should be rearranged into CH=O and N=N−OH yielding **3a**, $CH_3N{=}NOH$, and acetaldehyde, **3b**. This rearrangement is indicated by the following transform:

Find:	OH−CH−N−N=O
Replace with:	O =CH N=N−OH

It should be noted that since this rearrangement is carried out automatically, the net effect of the original hydroxylation is actually N-dealkylation.

Further consultation of the spontaneous dictionary will indicate that **3a** is still inherently unstable, and another

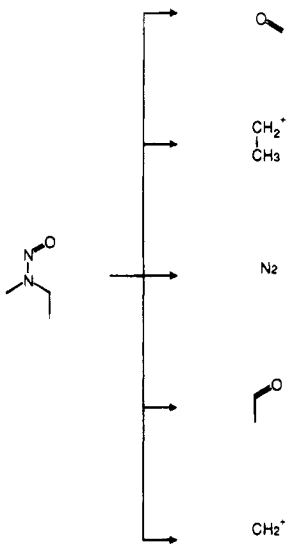

Figure 2. Metabolites of methylethyl-*N*-nitrosamine as produced by the META program.

transform is used to produce **4a**, CH_3^+; **4b**, N_2; and **4c**, H_2O. The process is repeated until no more unstable products are detected. The processing of spontaneous reactions usually operates in the background, and only the stable products are displayed. However, intermediates can be displayed on demand. Upon request for further transformation, the program will evaluate any one of the products for further processing and will continue to build the metabolic tree until it is determined that the final products can be excreted.

The program keeps a history of all the metabolic steps taken and will upon demand identify the enzyme class, relevant literature references, and the transform used to generate any of the metabolites. Duplications are eliminated from the compilation by comparing every new metabolite to those already on record so as to simplify the reporting process as much as possible.

Figure 1 shows the metabolic fate of methylethyl-*N*-nitrosamine when the ethyl branch is initially hydroxylated. Since the ethyl and methyl groups can be hydroxylated using slightly different transforms, the analogous products of methyl hydroxylation are also produced by the program as shown in Figure 2. As can be seen, carbonium ions, CH_3^+ and $CH_3CH_2^+$, are seen to result from this process. Consulting a third dictionary of fragments known to elicit biological activity, in this case, structural alerts of genotoxicity, the program will issue a warning that the initial molecule may be carcinogenic because it produces a metabolite suspected to be conducive to cancer.

Operation Support. Operation support is needed to address a number of problems encountered in the handling of the molecules generated by a computer automated manipulation of their structure. We have identified at least three problems that had to be resolved in order to support the operation of the META program.

The Ability To Recognize Unstable Molecules and the Need To Transform the Corresponding Molecular Structure. We have already alluded to this problem and mentioned the need for a dictionary of spontaneous reactions. For example, if by some metabolic hydroxylation, a hydroxyl group is attached to a carbon atom already bonded to another hydroxyl group, the program must be able to recognize that the molecule is basically unstable and will have to undergo

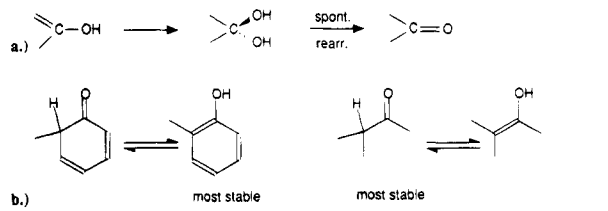

Figure 3. (a) Example of a spontaneous reaction. Transforms describing these reactions are collected into a separate dictionary used to process unstable intermediates. (b) Examples of tautomerism which require the use of quantum mechanics to complement structural transforms.

a spontaneous rearrangement, as shown in Figure 3a. A subsequent article will describe this dictionary in greater detail.

In some cases, the presence of the spontaneous dictionary is not sufficient, and it is necessary to evaluate more precisely the stability of related structures. An example of this situation exists for the nontrivial keto–enol tautomerism where subtle differences in the stability of two tautomers has to be evaluated as illustrated in Figure 3b.

In order to handle such problems, a simple quantum mechanical procedure was included in the META program. This routine automatically evaluates the stability of the isomers as required and invokes the transform that will generate the most stable product.

Effect of Solubility on the Probability of a Molecule To Encounter a Specific Enzyme. An example of this problem is seen in the action of P-450 which tends to exist in a lipophilic surrounding. Thus a molecule with low lipophilicity has a lower probability of being metabolized by such enzyme.

This problem was resolved by supplying the program with a log *P* (octanol/water partition coefficient) estimation routine. The LOGP program, which was published in a previous article,[13] calculates the partition coefficient of every molecule submitted to or generated by the META program. These values are made available to the routing unit for evaluation. Depending on this value, the routing unit will submit the molecule to the relevant transforms produced by enzymes compatible with the lipophilicity of the molecule. It will also, based on this value, assess the ability of the molecule to cross membranes or to move from one organ to another.

Relative Sensitivity of Several Sites of a Given Molecule to the Action of an Enzyme. This is of particular importance when assessing the potential metabolites of aromatic molecules where a number of delocalized double bonds may be candidates for oxidation by, say, cytochrome P-450. An example of this is the metabolism of phenanthrene as shown in Figure 4.

Here again, quantum mechanical techniques can be helpful. Some parametrization is required to fit the observed selectivity of the enzyme.

Quantum Mechanical Method. Since the three-dimensional geometry of the molecules submitted to the program is not generally available and its determination would be extremely time consuming, the possibility of using a simple connectivity based quantum mechanical method was explored. This turned out to be quite suitable because most of the problems encountered deal with relatively simple conjugated moieties. In most cases, a simple Hückel type method provides sufficient information to handle the prob-

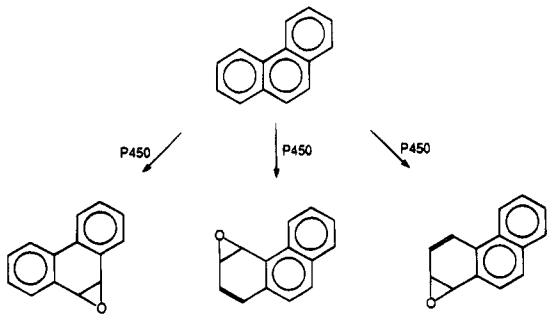

Figure 4. Phenanthrene metabolism.

lems discussed above. Van Catledge's parametrization, which is self-consistent, gives good results for this purpose.[14]

Oxidation of Aromatic Molecules. For the oxidation of aromatic molecules, an index had to be generated to help decide which bond, or atom, will be the most likely target for the metabolizing enzyme. We found that a satisfactory way of performing this task is to assess the nucleophilic character of each of the conjugated double bonds. We selected a procedure based on our previous description of charge and orbital control,[15] whereas the ability of a bond to undergo a concerted nucleophilic addition is mostly determined by its contribution to the HOMO of the molecule. The following nucleophilic index N was therefore defined for each bond

$$N_{xy} = 2\sum_{i_{occ}} \frac{(c_{ix} + c_{iy})^2}{0.1 + E_i}$$

where c_{ix} and c_{iy} are the coefficients of the atomic orbital of the bonded atoms x and y in the occupied molecular orbital i. E_i is the energy of the molecular orbital i. The advantage of this index is that it incorporates the orbital symmetry constraints needed to evaluate the potential for concerted addition to the bond.

Stability and Reactivity of Conjugated Systems. We also compute and store the more traditional charge densities Q_x and bond orders B_{xy} as follows:

$$Q_x = 1 - 2\sum_{i_{occ}} c_{ix}^2 \qquad B_{xy} = 2\sum_{i_{occ}} (c_{ix}c_{iy})$$

These indices can be used to assess the stability and reactivity of conjugated hydrocarbon moieties. For example, we found that the bond order of the double bond of an enol provides enough information to decide whether the enol is stable or is transformed into the keto form. Of the 18 enols we evaluated, those with a bond index less than 0.95 are stable as enols, while the rest are more stable in their keto form.

Management of Metabolites. A straightforward application of transforms such as illustrated in Figure 2 will yield, even for very simple molecules, a large number of possible products. Furthermore, with the ability to perform multiple metabolic steps, each of the products generated from the parent structure is available for further metabolism, resulting in more products. To manage the mass of information generated by the program, the following operations are performed.

First, a prioritization of the products by pharmacological significance identifies the major and minor products. Unless otherwise requested, minor products are not generated thereby reducing the number of metabolic pathways. This helps control the problem to some extent.

Second, duplicate structures at each metabolic step are eliminated. The recognition of duplicates is done by storing and comparing some graph invariants of increasing complexity. First a simple screen using the total number or atoms and the number of hydrogens is performed. If there is a match with a previously encountered structure, then the molecular weights are compared. If there is still a match, the determinant of a modified adjacency matrix is compared. The modification to the standard adjacency matrix is to use an index for each atom representing the atom type, hybridization, and number of non-hydrogen ligands, e.g., $CH_3(sp^3) = 4$, $CH_2(sp^3) = 5$, $CH(sp^3) = 6$, $C(sp^3) = 7$, $CH_2(sp^2) = 8$, etc. as the diagonal element. This method is effective in detecting and eliminating duplicates from the potentially large number of structures generated by the program.

Third, we use a criterion for terminating further biotransformations. Essentially, each product is evaluated for excretion by the kidneys. At each metabolic step, the octanol/water partition coefficients for the new metabolites are estimated. Compounds with values under a certain limit are presumed to be eliminated. We have a number of programs capable of estimating log P for diverse molecules,[13,16] and these are used in conjunction with this task.

And fourth, the development of the numerous transforms includes the cataloging of sample reactions and literature references. These data will be made available by the program on demand. Hence, the applicability of a given transform to a particular situation may be evaluated by the user.

DISCUSSION OF THE RESULTS

Imipramine, a commercially important antidepressant drug, provides a good example of the potential complexity and extent of xenobiotic biotransformation as illustrated in Figures 5 and 6. Its metabolites display a remarkable spectrum of physicochemical and pharmacological properties. Imipramine has become the classic model for the study of complex drug metabolism situations and their pharmacokinetic and pharmacodynamic implications.[17] Differences in the metabolism of imipramine are a major cause of variability in clinical response among individuals.[18]

Figure 5 shows the major primary routes of metabolism of this relatively simple molecule in mammals. A large fraction of a dose of imipramine is *N-dealkylated* to the therapeutically active product desipramine by the cytochrome P-450 system.[19] Imipramine undergoes *aromatic hydroxylation* to 2-OH-imipramine, which produces high cardiac toxicity and is responsible for teratogenic effects in rabbits.[20] Toxicity effects are of particular concern because depressed patients have a propensity to overdose their medication. Imipramine is also *N-oxidized* by the hepatic flavoprotein, amine oxidase, to imipramine-*N*-oxide.

There are notable interspecies differences in the metabolism of imipramine.[21-24] N-Oxidation is the major initial reaction in pigs, while N-dealkylation predominates in rats and guinea pigs. Desipramine is rapidly metabolized in mice and rabbits but accumulates in rats and humans.

As shown in Figure 6, the metabolic profile for imipramine expands broadly as secondary and subsequent pathways are

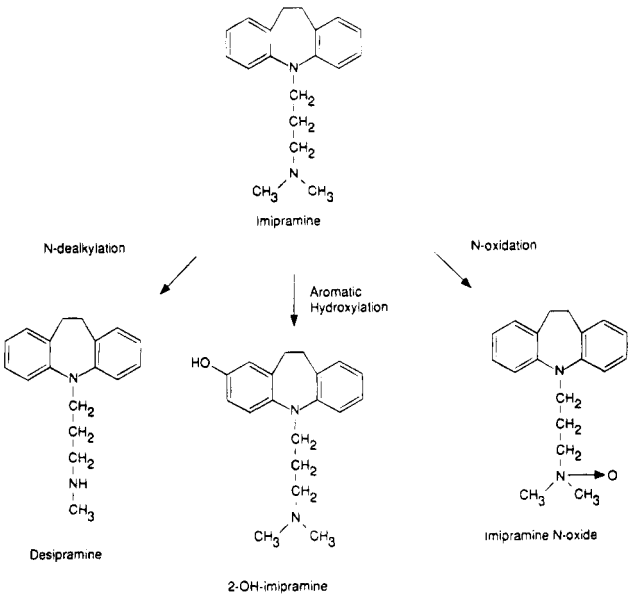

Figure 5. Major primary metabolic pathways of imipramine.

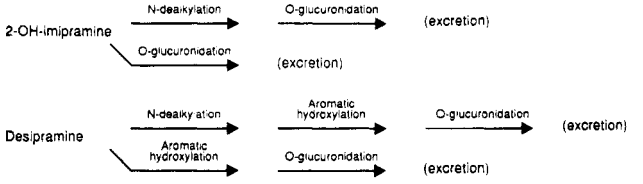

Figure 6. Some major secondary metabolic pathways of imipramine.

considered. Desipramine undergoes N-dealkylation and aromatic hydroxylation. The production of amine and alcohol functionalities in phase I metabolism allow *O-glucuronidation* in phase II metabolism, the products of which are the major excretion components in urine.[25-27] Figure 6 is not complete in that minor secondary metabolites are not shown. Indeed, more than 24 different metabolites have been detected and identified.

It is interesting to note that the N-dealkylation transform described earlier in this paper for methylethyl-*N*-nitrosamine also applies to imipramine. This same transform can remove the side chain of desipramine. Furthermore, a minor variant of this transform, i.e., the recognition of CH_3N as well as CH_2N as potential hydroxylation targets, can be used for the demethylation of both imipramine and 2-OH-imipramine. N-Dealkylation is one example of many META transforms that powerfully generalizes to apply to many different chemical structures.

Despite the complexity of imipramine metabolism, the major metabolic pathways of imipramine can be modeled successfully by only a few META transforms that correspond to the four biotransformations discussed above. These transforms may hit sequentially in various combinations, producing more than 30 distinct structures. However, some combinations, such as hydroxylated *N*-oxide metabolites, have never been experimentally observed. This points to the need to carefully design the transforms to ensure that only relevant metabolic biotransformations are predicted.

The metabolism of imipramine provides a good yardstick for the evaluation of META performance. The complex metabolism of molecules such as imipramine will be used to determine the predictive accuracy of META and guide the development of the dictionary. META acquires "intelligence" by developing transforms for specific biotransformations that will apply (even unexpectedly) to new biotransformations.

CONCLUSION

The breadth of this incomplete metabolic profile for a single simple molecule (Figure 6) highlights the formidable task of presenting a comprehensive listing of potential metabolites of environmentally important chemicals and drugs.

The computerized approach described in this paper is intended to make it possible not only to review the sequential formation of all conceivable metabolites from a given precursor molecule but also to scrutinize the relative importance of the various metabolites on the basis of priority rankings. These priorities can be developed according to relative abundance, tissue selectivity, isozyme selectivity, species variation, etc. The methodology dealing with assigning priorities will be described in an imminent publication.

The predictive power of the program is predicated on the breadth of the information that is provided in the dictionary. Thus a comprehensive data base from the literature is required.[28] It will be important to consider not only the bioactivities of the individual metabolites but also the potential for interactions (enhancement or inhibition of bioactivity) among the metabolites and the parent chemical agent or other chemical agents and their metabolites in cases of coincident exposure. The commitment to this tertiary level of utility of the program requires extensive development and thorough literature research as well as direct experimental verification. We will report on this aspect of the problem in a forthcoming publication.

REFERENCES AND NOTES

(1) Spann, M. L.; Chu, K. C.; Wipke, W. T.; Ouchi, G. Use of Computerized Methods to Predict Metabolic Pathways and Metabolites. *J. Environ. Pathol. Toxicol.* **1978**, *2*, 123.
(2) Wipke, W. T.; Ouchi, G. I.; Chou, J. T. In *Structure-Activity Correlation as a Predictive Tool in Toxicology*; L. Goldberg, Ed.; Hemisphere: New York, 1983; pp 151-169.
(3) Tinker, J. F.; Gelernter, H. Computer Simulation of Metabolic Transformation. *J. Comput. Chem.* **1986**, *7*, 657-665.
(4) Johnson, M. A.; Gifford, E.; Tsai, C.-c. In *Concepts and Applications of Molecular Similarity*; M. A. Johnson, Ed.; John Wiley & Sons, Inc.: New York, 1990; pp 289-320.
(5) Mavrovounitis, M. L.; Stephanopoulos, G.; Stephanopoulous, G. Computer-Aided Synthesis of Biochemical Pathways. *Biotechnol. Bioeng.* **1990**, *36*, 1119-1132.
(6) Darvas, F. Predicting metabolic pathways by logic programming. *J. Mol. Graphics* **1988**, *6*, 80-86.
(7) Corey, E. J.; Wipke, W. T. Computer-Assisted Design of Complex Organic Syntheses. *Science* **1969**, *166*, 178-192.
(8) Gelernter, H.; Sanders, A. F.; Larsen, D. L.; Ararwal, K. K.; Boive, R. H.; Spritzer, G. A.; Searleman, J. E. Empirical Explorations of SYNCHEM. *Science* **1977**, *197*, 1041-1049.
(9) Varkony, T. H.; Smith, D. H.; Djerassi, C. Computer-Assisted Structure Manipulation Studies in the Biosynthesis of Natural Products. *Tetrahedron* **1978**, *34*, 841-852.
(10) Klopman, G. Artificial Intelligence Approach to Structure-Activity Studies. Computer Automated Structure Evaluation of Biological Activity of Organic Molecules. *J. Am. Chem. Soc.* **1984**, *106*, 7315-7320.
(11) Klopman, G.; McGonigal, M. Computer Simulation of Physical Chemical Properties of Organic Molecules. *J. Chem. Inf. Comput. Sci.* **1981**, *21*, 48.
(12) Weininger, D. SMILES, a Chemical Language and Information Systems. *J. Chem. Inf. Comput. Sci.* **1988**, *28*, 31-36.
(13) Klopman, G.; Wang, S. A Computer Automated Structure Evaluation (CASE) Approach to the Calculation of Partition Coefficients. *J. Comput. Chem.* **1991**, *12*, 1025-1032.

(14) van Catledge, F. A. A Pariser-Parr-Pople-Based Set of Huckel Molecular Orbital Parameters. *J. Org. Chem.* **1980**, *45*, 4801−4802.
(15) Klopman, G. Chemical reactivity and the concept of charge and frontier-controlled reactions. *J. Am. Chem. Soc.* **1968**, *90*, 223−234.
(16) Klopman, G.; Namboodiri, K.; Schochet, M. Simple Method of Computing the Partition Coefficient. *J. Comput. Chem.* **1985**, *6*, 28−38.
(17) Bickel, M. H. In *Handbook of Experimental Pharmacology*; Hoffmeister, F., Stille, G., Ed.; Springer-Verlag: Berlin, 1980; pp 551−572.
(18) Gram, L. F. Plasma level monitoring of tricyclic antidepressant therapy. *Clin. Pharmacokinet.* **1977**, *2*, 237−251.
(19) Brosen, K.; Zeugin, T.; Meyer, U. A. Role of P450IID6, the target of the sparteinedebrisoquin oxidation polymorphism, in the metabolism of imipramine. *Clin. Pharmacol. Ther.* **1991**, *49*, 609−17.
(20) Aeppli, L. Teratologic Studies on Imipramine in Rats and Rabbits. A contribution to layout and interpretation of teratologic studies with special consideration of biochemistry and toxicology of the test substance. *Arzneim. Forsch.* **1969**, *19*, 1617−1640.
(21) Dingell, J. V. Species differences in the metabolism of imipramine and desipramine. *J. Pharmacol. Exp. Ther.* **1964**, *143*, 14−23.
(22) Gigon, P. L.; Bickel, M. H. N-demethylation and N-oxidation of imipramine by rat and pig liver microsomes. *Biochem. Pharmacol.* **1971**, *20*, 1921−1931.
(23) Bickel, M. H. N-oxide formation and related reactions in drug metabolism. *Xenobiotica* **1971**, *1*, 313−319.
(24) Quinn, G. P.; Hurwic, M. J.; Perel, J. H. In *Psychotherapeutic Drugs*; Usdin, E., Forrest, I. S., Ed.; Marcel Dekker: New York, 1976; pp 605−623.
(25) Christiansen, J.; Gram, L. F.; Kofod, B.; Rafaelsen, O. J. Imipramine metabolism in man. A study of urinary metabolites after administration of radioactive imipramine. *Psychopharmacologia* **1967**, *11*, 255−264.
(26) Crammer, J. L.; Scott, B.; Woods, H.; Rolfe, B. Metabolism of ^{14}C-Imipramine I. Excretion in the Rat and Man. *Psychopharmacologia* **1968**, *12*, 263−277.
(27) Crammer, J. L.; Scott, B.; Rolfe, B. Metabolism of 14C-Imipramine II. Urinary Metabolites in Man. *Psychopharmacologia* **1969**, *15*, 207−225.
(28) *Biotransformations*; Hawkins, D. R., Ed.; The Royal Society of Chemistry: London, 1988; Vol. 1.