MOLECULAR PHARMACOLOGY, 23:213–218

Quantitative Structure-Activity Study on the Mechanism of Inhibition of Microsomal *p*-Hydroxylation of Aniline by Alcohols

Role of Steric Factors

ALEKSANDAR SABLJIĆ[1] AND MIROSLAVA PROTIĆ-SABLJIĆ[2]

Laboratory of Chemical Physics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, and Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Received June 24, 1982; Accepted October 1, 1982

SUMMARY

Alkyl alcohols in millimolar concentrations competitively inhibit the microsomal *p*-hydroxylation of aniline. Two possible sites of inhibition have been proposed. Cohen and Mannering [*Mol. Pharmacol.* **9**:383–397 (1973)] suggested that alcohols bind to the active site of the free enzyme, the same site where aniline binds. More recently, Testa [*Chem. Biol. Interact.* **34**:287–300 (1981)] proposed an alternate mechanistic model, in which alcohols bind as ligands to the high-spin cytochrome P-450-aniline complex. Thus, it is important to determine which model is correct. In this paper, molecular connectivity indices were calculated for 20 alkyl alcohols and used as steric parameters in a quantitative structure-activity relationship (QSAR) study of the inhibitory activity of alkyl alcohols. A very good correlation ($R^2 = 0.967$) was obtained between the pIC_{50} values of these alcohols (negative logarithm of the concentration that causes 50% inhibition) and the valence zero-order and the fourth-order path/cluster molecular connectivity indices. This correlation was then used to predict the pIC_{50} values of 22 alkyl alcohols whose inhibitory activities have not as yet been experimentally determined. From our QSAR analysis, the interaction between cytochrome P-450 and the alkyl alcohols, which causes inhibition of aniline microsomal *p*-hydroxylation, can be viewed as a two-stage mechanism. The first stage is a "bond formation" between the hydroxyl group of alcohol and some site on the enzyme. The competitive character of the enzyme inhibition clearly shows that the binding of alcohols to cytochrome P-450 must take place at the active site of the free enzyme. The second stage is a hydrophobic interaction between the alkyl chain of the alcohol and a hydrophobic region of the enzyme that is close to the active site. It is assumed that this stage proceeds by the "zipper" mechanism and that it accounts for the quantitative differences in inhibition found for the studied alcohols.

INTRODUCTION

Cytochrome P-450 is the part of the cell's detoxification system which processes many foreign substances prior to their elimination. The binding of a variety of substances to the oxidized form of cytochrome P-450 is manifested as a change in the spectrum of the cytochrome (1, 2). On the basis of the shape of their difference spectra, compounds have been classified as Type I, Type II, and Reverse Type I (2). All Type I compounds are substrates. They bind to the active site of the enzyme (2) and range from hydrophobic to hydrophilic compounds. Binding of these substrates at the active site promotes the displacement of one axial (the sixth) ligand, causing the P-450 iron to be pentacoordinated (high-spin). Type II compounds, which generally contain accessible lone-pair electrons, bind *in vitro* to the high-spin heme iron in the sixth coordinate position (2), producing the low-spin form. Only a few Type II compounds are known to also be substrates of cytochrome P-450, and aniline is one of those (1). Reverse Type I compounds, which include alcohols, cause a spectral change which has been suggested (3) to be the reversal of the Type I spectral change.

Currently, two conflicting models have been proposed

This work was supported in part by the Self-Management Community of Interest for Scientific Research of S. R. Croatia (Yugoslavia).

[1] Laboratory of Chemical Physics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. Permanent address, Theoretical Chemistry Group, Department of Physical Chemistry, Institute Rudjer Bošković, 41001 Zagreb, Croatia, Yugoslavia.

[2] Laboratory of Molecular Carcinogenesis, National Cancer Institute. Permanent address, Laboratory for Marine Molecular Biology, Center for Marine Research Zagreb, Institute Rudjer Bošković, 41001 Zagreb, Croatia, Yugoslavia.

0026-895X/83/010213-06$02.00/0

to explain the inhibitory effect of alcohols on microsomal *p*-hydroxylation of aniline. In 1973, Cohen and Mannering (4) investigated the inhibitory activity of 22 alcohols. From their experimental results and a preliminary QSAR[3] study, they concluded that aniline *p*-hydroxylation was inhibited competitively by all of the primary alcohols and that the inhibitory potency of these alcohols increased with increasing carbon chain length. The potency of secondary and branched carbon chain alcohols as inhibitors was markedly less than that of the isomeric straight-chain primary alcohols. Cohen and Mannering (4) suggested that the first step in the mechanism of inhibition of aniline *p*-hydroxylation was the formation of a hydrogen bond between the hydroxyl group of the alcohol and some site of the enzyme, possibly the active site where the amino group of aniline binds. After hydrogen bond formation, the hydrophobic interaction between the alcohol carbon chain and some nonpolar site on the enzyme becomes important. Steric features of the alkyl chain were concluded to be decisive for this hydrophobic interaction. However, neither Taft's steric parameter E_s nor Hancock's corrected steric substituent E_s^c, which were used in Cohen-Mannering's QSAR study, adequately describe the influence of the branching of an alkyl chain on the potency of the inhibitory alcohols.

More recently, Testa (5) made a more comprehensive QSAR study on the structural and electronic factors influencing the inhibition of aniline *p*-hydroxylation by alcohols. The logarithm of the 1-octanol/water partition coefficient (log P) was found to be the structural parameter that gave the best correlation with inhibition in a bilinear equation. The log P values were calculated according to Rekker and de Kort (6) by adding the values of hydrophobic fragmental constants. The electronic parameters that, when coupled with steric parameters, gave the best correlations are the energies of the HOMO and the LUMO molecular orbitals. They were calculated by the semiempirical quantum mechanical MNDO method (7), using standard geometries of alcohols as input data. It is unfortunate that standard geometries were used with such a highly sophisticated semiempirical quantum mechanical method instead of optimized or experimental ones. On the basis of the results of his QSAR study, Testa proposed a mechanism to explain the inhibitory action of alcohols. He suggested that the alcohols bind as ligands to the high-spin cytochrome P-450-aniline complex, and the interaction between the alcohols and ferricytochrome P-450 was described as an electron exchange complex. In addition, he suggested that the alkyl chain of the alcohols binds to the hydrophobic and narrow cleft and that only the first carbon atoms along the chain (those which are closer to the hydroxyl group) contribute to the binding to the cleft.

There are thus two models proposed to explain the inhibitory effect of alcohols on microsomal *p*-hydroxylation of aniline, one in which the alcohol binds to the active site on cytochrome P-450 and one in which the alcohol binds as a ligand to the iron in cytochrome P-450. To help resolve this controversy we have carried out a quantitative structure-activity analysis of the steric factors influencing the inhibition of the microsomal *p*-hydroxylation of aniline by alcohols. This investigation reflects our continuous interest in the role of topological indices in structure-activity correlation studies (8–11). The steric parameters used in our QSAR analysis were calculated by the molecular connectivity method (8, 12, 13). This method provides a simple and accurate means for calculating the appropriate steric parameters which are the key factors for a meaningful QSAR study on the inhibition of aniline *p*-hydroxylation by alcohols. The results of our study should further enable us to deduce the position and mechanism of inhibition.

[3] The abbreviations used are: QSAR, quantitative structure-activity relationship; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital.

METHOD OF CALCULATION

The molecular connectivity method is based on molecular topology. The structural formula of a chemical compound is viewed as a molecular graph, where the vertices represent atoms and the edges represent the covalent bonds. Each molecular graph may be represented by a matrix, a polynomial, a sequence of numbers, or a numerical index. A numerical index characterizing a molecule is called a topological index (8, 12, 13). The topological indices express numerically, in a more or less discriminating manner, topological information about a given chemical structure. Topological information usually gives a "hint" about molecular size and shape. This method has the advantages of relative simplicity and flexibility. It can be used to represent molecular structure quantitatively at a number of levels of complexity. Each level provides some information directly related to the molecular structure and, through it, to physical, chemical, and biological characteristics. The connectivity index, χ, is currently the most tested of all topological indices in quantitative comparisons with physical, chemical, and biological properties of molecules in structure-property and structure-activity studies (8, 12, 13). Excellent correlations have been obtained between various connectivity indices and water solubility, octanol-water partition coefficient, boiling point, liquid density, or heat of vaporization (12). They have also been able to account for a great deal of the anesthetic activity of gases (14) and local anesthetics (12), the hallucinogenic activity of amphetamines (15), the activity of neurotransmitter agonists and antagonists (16, 17), and the mitodepressant activity of isatines (9). Extensive QSAR studies of enzyme inhibitors have been accomplished through the use of the topological indices (12, 18, 19). Simple linear correlations between the molecular connectivity index and the actions of inhibitors of succinate oxidase, thymidine phosphorylase, adenosine deaminase, and butyrylcholinesterase have been obtained (12). Detailed QSAR studies on hydrazide monoamine oxidase (18) and ribonucleotide reductase (19) have also been carried out by means of the topological indices. Furthermore, a linear relationship has been found between the connectivity index and the conversion of cytochrome P-450 to cytochrome P-420 in rabbit liver by a series of phenols (12). Most recently, bioconcentration factors (10) and soil sorption coefficients (11) of organic pollutants have been successfully correlated with the connectivity indices.

The concept of connectivity index, χ, was introduced

by Randić (20) and further developed and extensively used by Kier and Hall (12). Only a brief description of the calculation of the molecular connectivity indices used in this study is given here; a detail description of the theory and calculation of topological indices is published elsewhere (8, 12, 13).

The zero-order valence molecular connectivity index, ${}^0\chi^v$, refers to zero-order subgraphs consisting of simple vertices (atoms). Each vertex is described by its valence *delta* value, δ^v, which is calculated from the expression, $\delta^v = Z^v - h$, where Z^v is the number of valence electrons in the atom (vertex) and h is the number of hydrogen atoms bound to the same atom. The index is then calculated for each compound according to the expression,

$$ {}^0\chi^v = \sum_{i=1}^{n} (\delta_i^v)^{-0.5} $$

where n is the number of non-hydrogen atoms in a molecule.

The second topological index used in the study is the fourth-order path/cluster molecular connectivity index, ${}^4\chi_{PC}$. It refers to subgraphs consisting of four adjacent edges (bonds), three of which are joined to the same central vertex (cluster). Each vertex is described by its *delta* value, δ, which is equal to the number of adjacent non-hydrogen atoms. This index is then calculated from the following expression:

$$ {}^4\chi_{PC} = \sum_{z=1}^{n} (\delta_i \cdot \delta_j \cdot \delta_k \cdot \delta_l \cdot \delta_m)_z^{-0.5} $$

where i, j, k, l, and m correspond to the individual vertices that form the subgraph, and the summation is over all fourth-order path/cluster subgraphs in a molecule.

Molecular connectivity indices were calculated by a CFUNC computer program (21), whereas single and multiple linear regression analyses were performed using a statistical analysis system. To test the quality of the regression equations, the following statistical parameters were used: single (r) and multiple (R) correlation coefficients, the standard error of the estimate (s), a test of the null hypothesis (F-test), and the amount of explained variance (EV). All calculations were carried out on a DECsystem10 computer at the National Institutes of Health.

RESULTS AND DISCUSSION

The alkyl alcohols examined in the present QSAR study are shown in Table 1 together with their inhibitory potency and topological indices used as structural descriptors.

The inhibitory activities of the alcohols are taken from the comprehensive study of Cohen and Mannering (4). Two compounds, benzyl alcohol and 1,1-dimethyl-1-propanol, have been omitted from the current QSAR analysis. Benzyl alcohol does not belong to the class of alkyl alcohols. Thus, it is statistically unjustifiable to take into analysis a type of structure that is represented by only one compound. The same is true for 1,1-dimethyl-1-propanol, since it is the only tertiary alcohol. Furthermore, a qualitatively different mode of interaction with cytochrome P-450 has been proposed (5) for the latter compound.

TABLE 1

Molecular connectivity indices and inhibitory activity of alcohols on the microsomal p-hydroxylation of aniline

Compound	${}^0\chi^v$	${}^4\chi_{PC}$	pIC_{50}[a]	
			Experimental[b]	Calculated[c]
Methanol	1.447	—	−3.09	−2.91
Ethanol	2.154	—	−1.10	−1.34
1-Propanol	2.861	—	−0.48	−0.55
1-Butanol	3.569	—	−0.05	−0.08
1-Pentanol	4.276	—	0.27	0.24
1-Hexanol	4.983	—	0.54	0.47
1-Heptanol	5.690	—	0.68	0.64
2-Methyl-1-propanol	3.732	0.408	−0.39	−0.46
2-Methyl-1-butanol	4.439	0.577	−0.15	−0.36
3-Methyl-1-butanol	4.439	0.289	−0.19	−0.03
2,2-Dimethyl-1-propanol	4.654	1.061	−0.67	−0.84
2-Propanol	3.025	—	−0.47	−0.42
2-Butanol	3.732	0.408	−0.35	−0.46
2-Pentanol	4.439	0.289	−0.07	−0.03
2-Hexanol	5.146	0.289	0.15	0.18
2-Heptanol	5.852	0.289	0.25	0.34
3-Pentanol	4.439	0.577	−0.37	−0.36
3-Hexanol	5.146	0.493	−0.47	−0.05
2-Methyl-3-pentanol	5.309	1.276	−0.89	−0.90
2,4-Dimethyl-3-pentanol	6.179	1.821	−1.38	−1.34

[a] Negative logarithm of alcohol concentration (millimolar) that causes 50% inhibition.

[b] From Ref. 4.

[c] Calculated by Eq. 2.

Preliminary single-variable regression equations were calculated for the zero- and first-order molecular connectivity indices of unbranched primary alcohols (methanol to 1-heptanol) as a test sample. This preliminary analysis was carried out in order to find an index that would most adequately describe the general characteristics of steric properties, which are usually proportional to the numbers and types of atoms and bonds. From the correlation diagrams it was easy to conclude that the hyperbolic relation is apparent between the connectivity indices and the inhibitory activities of the alcohols. The best one-variable equation was obtained for the valence zero-order molecular connectivity index, ${}^0\chi^v$,

$$ pIC_{50} = -(7.16 \pm 0.28)/{}^0\chi^v + (1.99 \pm 0.11) \qquad (1) $$

$$ n = 7 \quad r = 0.996 \quad s = 0.126 \quad F^{1,5} = 647. \quad EV = 0.99 $$

The statistical analysis demonstrates an excellent correlation between the valence zero-order molecular connectivity index and the inhibition effect of alcohols. Equation 1 accounts for 99% of the variation in the pIC_{50} data. The standard error(s) of the estimate is less than 4% of the range of pIC_{50} values, and the equation is statistically significant above the 95% level [$F = 647. > 230.2 = F(1,5,0.5)$]. The 95% confidence intervals are shown in parentheses. When secondary and branched alcohols were included in the regression analysis, a single-variable equation was able to account for only 60% of the variation of the inhibition data. This is not surprising, since low-order molecular connectivity indices are not

capable of describing the fine details of branching. Therefore, our next objective was to find a higher-order connectivity index that would correctly reflect the relation between the position and type of branching of the alkyl chain and the hydroxyl group. The fourth-order path/cluster connectivity index, $^4\chi_{PC}$, was examined first, since it has been found to be important in predicting molecular volume (21). Combined with the $^0\chi^v$ index, it yielded the best two-variable equation. Five other higher-order cluster and path/cluster indices were also tested in two-variable equations but were found to be less successful. The best two-variable equation and its statistical parameter are as follows:

$$pIC_{50} = -(6.88 \pm 0.32)/^0\chi^v - (1.14 \pm 0.08)\cdot{}^4\chi_{PC} + (1.85 \pm 0.11) \quad (2)$$

$$n \equiv 20 \qquad R^2 \equiv 0.967 \qquad s \equiv 0.156 \qquad F^{2,17} \equiv 249. \qquad EV \equiv 0.963$$

Equation 2 accounts for 96.3% of the variation in the pIC_{50} data, and the standard error of the estimate is only 4% of the range of the pIC_{50} data. The statistical significance of Eq. 2 is above the 99.5% level ($F \equiv 249. \geq 199.4 \equiv F(2,17,0.005)$), while both variables are significant above the 99% level (Student's t-test). The magnitude of the correlation coefficient between the two variables ($r^2 \equiv 0.174$) indicates that there is no apparent cross-correlation that will prohibit their simultaneous presence in a regression equation. Three-variable equations were not examined, since Eq. 2 already accounts for more than 95% of the variation in pIC_{50} data and the error in pIC_{50} data exceeds the residual 5% variation. In our regression analysis, 10 variables were screened for 20 observations. Topliss and Edwards (22) studied the relationship between the number of observations and the number of examined variables from simulated correlations using sets of random numbers. It was demonstrated that, in order to investigate 10 variables, it is necessary to have at least 16 observations ($R^2 \geq 0.9$) if the probability of chance correlation is to be less than 1%. Thus, for 20 observations and $R^2 \equiv 0.967$, the probability of chance correlation is well below 1%.

Because of its statistical significance, Eq. 2 has been used to calculate the pIC_{50} values for the alkyl alcohols whose inhibitory activities have not as yet been measured. Results are presented in Table 2. The alcohols with up to seven carbon atoms in the alkyl chain were selected for this calculation, because of the insolubility of the higher alcohols in the aqueous medium (4). The experimental examination of the selected alkyl alcohols (Table 2) should afford a constructive challenge to the molecular connectivity approach.

The present QSAR analysis shows that the degree of inhibition of aniline p-hydroxylation by alcohols is greatly influenced by the size and type of branching of the alkyl chain. The negative regression coefficient of the $^0\chi^v$ index and its reciprocal (hyperbolic) relationship to inhibition leads to the conclusion that inhibition increases with the size of the molecule, since the numerical value of the $^0\chi^v$ index is directly proportional to the number of atoms in a molecule. The hyperbolic relationship also indicates that each new increment of the alkyl chain length increases potency less than the previous one and that at a certain length a plateau will be reached. On the other hand, the negative regression coefficient of the $^4\chi_{PC}$ index indicates that branching lowers the inhibitory activity of the alcohols. The $^4\chi_{PC}$ index is highly sensitive to changes in branching, and its value rapidly increases with the degree of branching. This index is capable of describing the influences of all variations in the first and second adjacent atoms from the branching point as well as all of the interactions along the four-atom chain. Thus the resulting inhibitory effect of the alcohols will be a fine balance between the size and the degree of branching of the alkyl chain. The $^4\chi_{PC}$ index was also found to be very useful in the case of the polysubstituted benzenes (23). It contains structural informations about the number of benzene-ring substituents, the substitution pattern, the length of the substituents up to three bond lengths, and the type of substituent.

From experimental measurements (4) and our QSAR analysis, the interaction between cytochrome P-450 and the studied alcohols, which cause inhibition of aniline microsomal p-hydroxylation, can be viewed as a two-stage mechanism. The first stage is a "bond formation" (hydrogen bond or polar interaction) between the hydroxyl group of the alcohol and some site on the enzyme. This stage is responsible for the inhibitory activity of the alcohols. The second stage is a hydrophobic interaction between the alkyl chain and a hydrophobic region that is close to the enzyme site involved in the first stage. This stage may account for the quantitative differences in activity found for the studied alcohols. Since the alkyl alcohols possess a high degree of conformational freedom, it is reasonable to assume that the binding to the enzyme will proceed by the "zipper" mechanism (24). In this mechanism, it is proposed that the initial interaction

TABLE 2

Predicted inhibitory activity (from Eq. 2) and molecular connectivity indices of the alkyl alcohols

For explanation see Table 1.

Compound	pIC_{50} (calc.)	$^0\chi^v$	$^4\chi_{PC}$
2-Methyl-1-pentanol	−0.05	5.146	0.493
3-Methyl-1-pentanol	−0.05	5.146	0.493
4-Methyl-1-pentanol	0.18	5.146	0.289
2,3-Dimethyl-1-pentanol	−0.75	6.016	1.276
2,4-Dimethyl-1-pentanol	−0.08	6.016	0.691
3,4-Dimethyl-1-pentanol	−0.67	6.016	1.207
2-Methyl-1-hexanol	0.11	5.853	0.493
3-Methyl-1-hexanol	0.21	5.853	0.408
4-Methyl-1-hexanol	0.11	5.853	0.493
5-Methyl-1-hexanol	0.35	5.853	0.289
3-Heptanol	0.11	5.853	0.493
4-Heptanol	0.21	5.853	0.408
3-Methyl-2-butanol	−1.16	4.602	1.333
3-Methyl-2-pentanol	−0.90	5.309	1.276
4-Methyl-2-pentanol	0.02	5.309	0.471
3,4-Dimethyl-2-pentanol	−1.34	6.179	1.821
3-Methyl-2-hexanol	−0.67	6.016	1.207
4-Methyl-2-hexanol	−0.08	6.016	0.691
5-Methyl-2-hexanol	0.05	6.016	0.577
2-Methyl-3-hexanol	−0.67	6.016	1.207
4-Methyl-3-hexanol	−0.75	6.016	1.276
5-Methyl-3-hexanol	−0.08	6.016	0.691

(first-stage) is followed by a series of conformational rearrangements of the partially bound alcohol, leading to the binding of the remaining segments of the alkyl chain to their appropriate positions. Such a mechanism allows mutual conformational adjustment of both alcohol and enzyme. The zipper mechanism has also been proposed as an explanation for the double-helix formation in nucleic acids and the receptor binding of peptide hormones (24) and for methadone binding to opiate receptor sites (25).

Aniline *p*-hydroxylation is inhibited competitively by the studied alcohols (4). The main characteristic of competitive enzyme inhibition is that the inhibitor combines with the free enzyme in such a way that it competes with the substrate for binding at its active site (26). Thus, it is evident that the binding of alcohols to cytochrome P-450, which causes the competitive inhibition of aniline *p*-hydroxylation, must take place at the active site of the free enzyme. The mechanistic model for the inhibition of aniline *p*-hydroxylation by alcohols as proposed by Testa (5), where the alcohols bind as ligands to the high-spin cytochrome P-450-aniline complex, clearly represents uncompetitive enzyme inhibition. In uncompetitive inhibition, the inhibitor combines with the enzyme-substrate complex to give an inactive enzyme-substrate-inhibitor complex, which cannot undergo further reaction to yield the product (26). It is highly surprising that the uncompetitive inhibition model was postulated from the kinetic measurements (4) of a competitive inhibition. In addition, it is most unlikely that alcohols, at least in millimolar concentrations, will bind as ligands, either to free or substrate-bound high-spin cytochrome P-450, in the presence of aniline since they have a much lower affinity for the ferric ion than aniline (4). Moreover, Ingelman-Sundberg and Johansson (27) and Ingelman-Sundberg and Ekström (28) recently suggested the same mechanism for microsomal oxidation of ethanol and *p*-hydroxylation of aniline. This is additional evidence that the inhibition of *p*-hydroxylation of aniline by alcohols represents the competition of substrates for the active site of enzyme.

Our results strongly suggest that the QSAR analysis made by Testa (5) suffers from very serious shortcomings. All isomers, alcohols which have the same number of carbon atoms in the alkyl chain, have the same calculated log P values, whereas the experimental values are considerably different (Table 3). Thus it was quite surprising that the parameter with such a low discriminating power could produce such a good correlation between log P and the inhibitory activity of the alcohols (R^2 = 0.929 for correlation with one additional steric parameter—total number of carbon atoms in a molecule divided by the number of carbon atoms in the main chain of the molecule). Moreover, Hansch *et al.* (29), Leo *et al.* (30), and Rekker and de Kort (6), who proposed the method for calculating log P, have always advocated the use of experimental log P values whenever accurate measurements are available, as is the case with alkyl alcohols (29, 30). Furthermore, the statistical significance of the two electronic parameters (the HOMO and LUMO energies), when included in the regression equation with the steric parameters, was only marginal. The correlations were improved by only 3% (R^2 = 0.965), and even this may not be meaningful because the IC_{50} data error is approximately 12%. [The range of the calculated HOMO energies was only 0.34 eV, their average energy was 11.31 eV, and the standard error of the HOMO's regression coefficient (0.381) was over 50%. Similar results were found for the LUMO parameter.] Thus, it may be concluded that the poor selection of parameters for the QSAR study made by Testa (5) resulted in an improbable model for inhibition of aniline microsomal *p*-hydroxylation by alcohols.

TABLE 3

Calculated and experimental log P values of some alkyl alcohols

Compound	N[a]	Log P	
		Calculated[b]	Experimental[c]
1-Propanol	3	0.27	0.34
2-Propanol	3	0.27	0.14
1-Butanol	4	0.79	0.88
2-Butanol	4	0.79	0.61
1-Pentanol	5	1.31	1.40
2,2-Dimethyl-1-propanol	5	1.31	1.36
2-Pentanol	5	1.31	1.14
1,1-Dimethyl-1-propanol	5	1.31	0.89
1-Hexanol	6	1.83	1.84
2-Hexanol	6	1.83	1.61
2-Methyl-3-pentanol	6	1.83	1.41
1-Heptanol	7	2.35	2.41
2-Heptanol	7	2.35	2.33
2,4-Dimethyl-3-pentanol	7	2.35	1.71

[a] Number of carbon atoms in a molecule.
[b] Calculated according to Rekker and de Kort (6).
[c] Experimental values are taken from refs. 29 and 30.

CONCLUSION

From our QSAR analysis and previous experimental data (4) it can be concluded that the binding of alcohols, which cause inhibition of aniline *p*-hydroxylation, take place at the enzyme-active site. The size and type of branching of the alkyl chain of the alcohols account for the quantitative differences in the inhibition. The structural parameters used in our QSAR study, viz., the molecular connectivity indices, adequately describe these quantitative differences. Our results are in agreement with the experimental work and model proposed by Cohen and Mannering (4).

ACKNOWLEDGMENTS

We would like to thank Dr. Ruth McDiarmid and Dr. Susan P. C. Cole for the very helpful discussions and suggestions made during the preparation of the manuscript.

REFERENCES

1. Remmer, H., J. B. Schenkman, R. W. Estabrook, H. Sasame, J. Gillette, S. Narsimhulu, D. Y. Cooper, and O. Rosenthal. Drug interaction with hepatic microsomal cytochrome. *Mol. Pharmacol.* 2:187–190 (1966).
2. Schenkman, J. B., H. Remmer, and R. W. Estabrook. Spectral studies of drug interaction with hepatic microsomal cytochrome. *Mol. Pharmacol.* 3:113–123 (1967).
3. Schenkman, J. B., H. Greim, M. Zange, and H. Remmer. On the problem of possible other forms of cytochrome P-450 in liver microsomes. *Biochim. Biophys. Acta* 171:23–31 (1969).

4. Cohen, G. M., and G. J. Mannering. Involvement of a hydrophobic site in the inhibition of the microsomal *p*-hydroxylation of aniline by alcohols. *Mol. Pharmacol.* **9**:383–397 (1973).
5. Testa, B. Structural and electronic factors influencing the inhibition of aniline hydroxylation by alcohols and their binding to cytochrome P-450. *Chem. Biol. Interact.* **34**:287–300 (1981).
6. Rekker, R. F., and H. M. de Kort. The hydrophobic fragmental constant: an extension to a 1000 data point set. *Eur. J. Med. Chem.* **14**:479–488 (1979).
7. Dewar, M. J. S., and W. Thiel. Ground states of molecules. 38. The MNDO method. Approximations and parameters. *J. Am. Chem. Soc.* **99**:4899–4907 (1977).
8. Sabljić, A., and N. Trinajstić. Quantitative structure-activity relationships: the role of topological indices. *Acta Pharm. Jugosl.* **31**:189–214 (1981).
9. Sabljić, A., N. Trinajstić, and D. Maysinger. Molecular connectivity and biological activity in a series of isatin derivatives. *Acta Pharm. Jugosl.* **31**:71–76 (1981).
10. Sabljić, A., and M. Protić. Molecular connectivity: a novel method for prediction of bioconcentration factor of hazardous chemicals. *Chem. Biol. Interact.* **42**:, in press (1982).
11. Sabljić, A., and M. Protić. Relationship between molecular connectivity indices and soil sorption coefficients of polycyclic aromatic hydrocarbons. *Bull. Environ. Contam. Toxicol.* **28**:162–165 (1982).
12. Kier, L. B., and L. H. Hall. *Molecular Connectivity in Chemistry and Drug Research.* Academic Press, New York (1976).
13. Balaban, A. T., A. Chiriac, I. Motoc, and Z. Simon. *Steric Fit in Quantitative Structure-Activity Relationships. Lecture Notes in Chemistry No. 15.* Springer-Verlag, Berlin (1980).
14. Di Paolo, T., L. B. Kier, and L. H. Hall. Molecular connectivity and structure-activity relationship of general anesthetics. *Mol. Pharmacol.* **13**:31–37 (1977).
15. Kier, L. B., and L. H. Hall. Structure-activity studies on hallucinogenic amphetamines using molecular connectivity. *J. Med. Chem.* **20**:1631–1636 (1977).
16. Kier, L. B., and L. H. Hall. Molecular connectivity study of muscarinic receptor of acetylcholine antagonists. *J. Pharm. Sci.* **67**:1408–1412 (1978).
17. Kier, L. B., and R. A. Glennon. Psychotomimetic phenalkylamines as serotonin agonists: an SAR analysis. *Life Sci.* **22**:1589–1593 (1978).
18. Richard, A. J., and L. B. Kier. Structure-activity analysis of hydrazide monoamine oxidase inhibitors using molecular connectivity. *J. Pharm. Sci.* **69**:124–126 (1980).
19. van't Riet, B., L. B. Kier, and H. L. Elford. Structure-activity relationships of benzohydroxamic acid inhibitors of ribonucleotide reductase. *J. Pharm. Sci.* **69**:856–857 (1980).
20. Randić, M. On characterization of molecular branching. *J. Am. Chem. Soc.* **97**:6609–6615 (1975).
21. Hall, L. H., and L. B. Kier. The relation of molecular connectivity to molecular volume and biological activity. *Eur. J. Med. Chem.* **16**:399–407 (1981).
22. Topliss, J. G., and R. P. Edwards. Chance factors in studies of quantitative structure-activity relationships. *J. Med. Chem.* **22**:1238–1244 (1979).
23. Kier, L. B. Structural information from molecular connectivity $^4\chi_{PC}$ index. *J. Pharm. Sci.* **69**:1034–1039 (1980).
24. Burgen, A. S. V., G. C. K. Roberts, and J. Feeney. Binding of flexible ligands to macromolecules. *Nature (Lond.)* **253**:753–755 (1975).
25. Klasinc, L., B. Ruščić, A. Sabljić, and N. Trinajstić. Application of photoelectron spectroscopy to biologically active molecules and their constituent parts. 6. Opiate narcotics. *J. Am. Chem. Soc.* **101**:7477–7482 (1979).
26. Lehninger, A. L. *Biochemistry*, Ed. 2. Worth Publishers, New York, 183–216 (1981).
27. Ingelman-Sundberg, M., and I. Johansson. The mechanism of cytochrome P-450-dependent oxidation of ethanol in reconstituted membrane vesicles. *J. Biol. Chem.* **256**:6321–6326 (1981).
28. Ingelman-Sundberg, M., and G. Ekström. Aniline is hydroxylated by the cytochrome P-450-dependent hydroxyl radical-mediated oxygenation mechanism. *Biochem. Biophys. Res. Commun.* **106**:625–631 (1982).
29. Hansch, C., J. E. Quinlan, and G. L. Lawrence. The linear free-energy relationship between partition coefficients and the aqueous solubility of organic liquids. *J. Org. Chem.* **33**:347–350 (1968).
30. Leo, A., C. Hansch, and D. Elkins. Partition coefficients and their uses. *Chem. Rev.* **71**:525–616 (1971).

Send reprint requests to: Dr. Aleksandar Sabljić, Laboratory of Chemical Physics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Building 2, Room B2-01, National Institutes of Health, Bethesda, Md. 20205.