further investigations of the behavior of particulate matter administered into the bloodstream.

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ACKNOWLEDGMENTS

Presented at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, New Orleans meeting, April 1976.

The work on which this report is based was supported by the Public Health Service, Food and Drug Administration, Department of Health, Education, and Welfare, Contract 223-77-3018.

The authors thank Dr. Daniel A. Weiss, Professor of Pathology, for his guidance and consultation in the histological evaluations.

Correlation of Log *P* with Molecular Connectivity in Hydroxyureas: Influence of Conformational System on Log *P*

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Abstract □ The correlation of log *P* (in octanol–water) with the nonempirical, topologically dependent, calculated molecular connectivity index ($^1\chi^v$) delineates substituted hydroxyureas into two families of linearly related groups of compounds. The first group, composed of the 3-substituted ethyl, *n*-propyl, and *n*-butyl analogs, is more hydrophilic than the 1-substituted methyl and ethyl and the 3-substituted isopropyl and *tert*-butyl analogs. The unsubstituted model compound hydroxyurea appears between the two groups in equal volumes of octanol. In octanol–water ratios of 5:1, log *P* approaches the range of the more hydrophilic group in high concentrations and becomes more lipophilic (similar to the other group) in lower concentrations. The differences in the relative hydrophilicities–lipophilicities of the two groups are rationalized in terms of the equilibria of internally hydrogen-bonded conformers to those that allow optimal interactions with solvent, water, or other hydroxyurea molecules. The concentration dependency observed with hydroxyurea appears to be due to the ease of interconversion of intermolecularly bonded conformers to those interacting with water, whereas the involvement of internally bonded conformers, which are apparently present to a greater degree in lower concentrations, increases the relative lipophilicity.

Keyphrases □ Hydroxyureas, various—log *P* in octanol–water correlated with molecular connectivity indexes □ Log *P*—various hydroxyureas in octanol–water, correlated with molecular connectivity indexes □ Molecular connectivity indexes—various hydroxyureas, correlated with log *P* in octanol–water □ Topological indexes—molecular connectivity, various hydroxyureas, correlated with log *P* in octanol–water

Many physicochemical properties are presently used in relating molecular structure to biological activities (structure–activity relationships) (1). The term physicochemical activity relationships has been suggested (2) to differentiate these methods from strictly structural approaches, *e.g.*, the Free–Wilson method, or from approaches that derive parameters from theoretical calculations on molecular structures, *e.g.*, quantum mechanical methods. The most extensively applied techniques are those that relate biological activity to free energy changes associated with drug transport through different environmental phases. Of the various methods of physicochemical or quantitative structure–activity relationships developed, Hansch analysis (3) has made significant contributions in the realm of quantitative structure–activity relationships and drug design, primarily because of the important role of transport and absorption in drug activity.

BACKGROUND

One primary function of Hansch analysis and other physicochemical and structure–activity methods is to predict optimal molecular or structural characteristics for the design of chemical agents to improve or optimize biological activity. Another utility of these methods might be to study the dynamics of drugs that have been synthesized, biologically tested, and evaluated for medicinal activity but for which there is not an accepted explanation for the activity or lack of biological activity of members of the class.

Table I—Observed Log *P* and Calculated Molecular Connectivity ($^1\chi^v$) of Selected Hydroxyureas of Various Tautomeric Forms which Represent Conformers Possessing Various Localizations of π-Bond Character

Compound	R_1	R_2	Log *P*	Calculated $^1\chi^v$ of Hydroxyurea Species R_1NCNOH (=O)	$R_1NC{=}NOH$ (–OH)	$R_1N{=}CNOH$ (–OH)
Hydroxyurea	H	H	−1.27	0.966	0.959	0.947
1-Methylhydroxyurea	H	CH_3	−0.46	1.364	—	1.344
1-Ethylhydroxyurea	H	C_2H_5	−0.10	1.940	—	1.921
3-Ethylhydroxyurea	C_2H_5	H	−0.76	1.988	1.958	1.944
3-*n*-Propylhydroxyurea	*n*-C_3H_7	H	−0.22	2.488	2.458	2.444
3-Isopropylhydroxyurea	iso-C_3H_7	H	0.20	2.371	2.458	2.334
3-*n*-Butylhydroxyurea	*n*-C_4H_9	H	0.32	2.988	2.958	2.944
3-*tert*-Butylhydroxyurea	*tert*-C_4H_9	H	0.50	2.678	2.647	2.644

A drug class that fits this description is the hydroxyurea class of antileukemia, anticancer agents of which the simplest member, hydroxyurea, is the most active (4). Molecular modification has not resulted in an analog of superior biological action (5), and no accepted explanation has come forth as to why minor substitutions on the hydroxyurea nucleus diminish biological action. Since the molecular mechanism of action of hydroxyurea remains unknown, the relative contributions of drug absorption, transport, concentration, and dynamics at the site of action have not been established. The method of Hansch has been applied to hydroxyureas (6) to evaluate the relative importance of drug transportability by comparison of the predicted partition coefficients (log *P*) (*via* the summing of fragment values) *versus* the observed log *P* values determined in octanol–water. Most log *P* values for the substituted hydroxyureas were more hydrophilic than predicted, with the greatest differences (Δ log *P*) in predicted *versus* observed values being in the 3-substituted straight chain analogs, *e.g.*, ethyl, *n*-propyl, and *n*-butyl (all Δ log *P* of 0.70). The differences in predicted *versus* observed log *P* for the 1-substituted and bulky 3-substituted analogs were much less, and the observed log *P* for hydroxyurea was much more lipophilic than predicted utilizing the Hansch method.

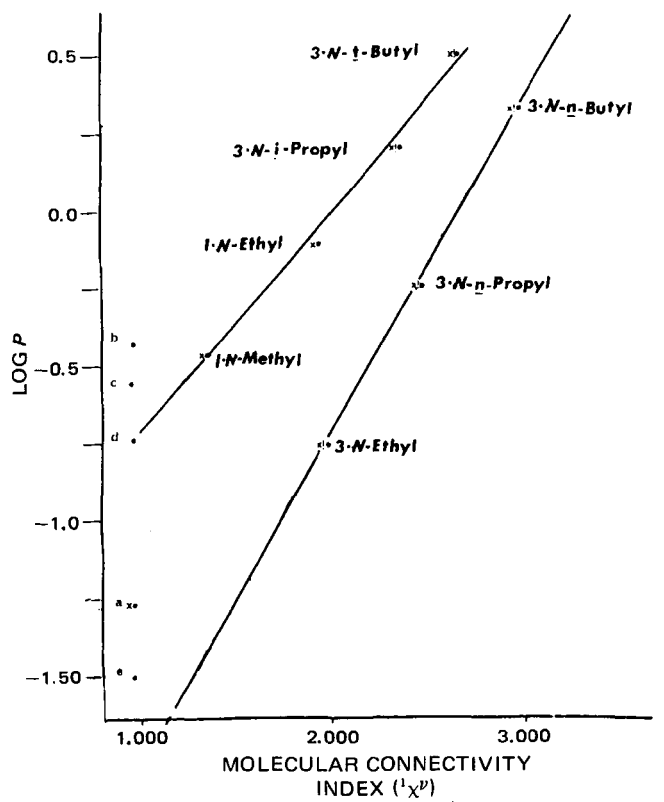


Figure 1—*Plot of log* P versus *valence molecular connectivity for selected hydroxyureas. Valence molecular connectivity is calculated for the* π*-bond at* C=O (*), C=N[1] (!), *and* C=N[3] (×). *Log* P *of hydroxyurea is shown at concentrations in octanol–water (1:1) (a) and in octanol–water (5:1) containing 0.1 (b), 0.5 (c), 1.0 (d), and 2.0 (e) g of solute.*

Log *P* was found recently to be related to the topological structure of a molecule, *e.g.*, the number and manner that atoms are arranged in a molecule (known as molecular connectivity) (7). The molecular connectivity index, χ, developed and utilized by Kier and coworkers (8, 9) and based on an earlier branching index by Randic (10), is a nonempirical, easily calculated value which seemingly encodes the shape and architecture of a molecule *via* the interatomic connections. The method of Kier and Hall, modified for the application to heteroatoms (valence connectivities) (8, 11, 12), was applied to the hydroxyurea series to determine if there was a correlation between log *P* and molecular structure as described by $^1\chi^v$.

EXPERIMENTAL

Hydroxyureas—Hydroxyurea[1], 1-methylhydroxyurea[1], and ethylhydroxyurea[1] were used as received. The 3-substituted analogs were synthesized according to the original procedure of Dresler and Stein (13) as modified (14, 15).

Log *P* Determinations—Log *P* values for the hydroxyureas were obtained as previously reported (6) by direct determination of the aqueous phase after equilibration for a minimum of 24 hr between octanol and water (pH 7). Log *P* was initially determined in equal volumes of water and octanol (50 ml of each) at concentrations containing 0.1, 0.5, and 1.0 g of hydroxyurea, with three determinations at each concentration. Determinations also were performed with five volumes of octanol to one volume of water, and only with unsubstituted hydroxyurea did the log *P* values vary appreciably.

Molecular Connectivity Index Calculation—The molecular connectivity was calculated according to the method of Kier and Hall as modified for molecules containing heteroatoms (8, 11, 12). The molecular skeleton was drawn, and each carbon atom in the skeleton was assigned a number, 1, 2, 3, or 4 (δ_i, δ_j), corresponding to the number of nonhydrogen atoms connected to the carbon atom. A number (δ_i^v, δ_j^v) was assigned to each heteroatom equivalent to the valence number minus the number of hydrogens connected to each heteroatom. A number was derived for each bond in calculating the product of the numbers associated with the two atoms of the bond. The reciprocal of the square root of this number was then computed and became the bond value. Finally, the bond values were summed to give a number, called the valence connectivity index ($^1\chi^v$) for the molecule; $^1\chi^v = \Sigma(\delta_i^v, \delta_j^v)^{-1/2}$.

For each hydroxyurea, three calculations were made: (*a*) for the molecule with a C=O bond at carbonyl carbon, (*b*) for a molecule with a C=N between the 1-nitrogen and carbonyl carbon, and (*c*) for a molecule with a N=C between the 3-nitrogen and carbonyl carbon.

The calculated connectivities ($^1\chi^v$) for each of these molecular species and the observed log *P* values for each of the hydroxyureas evaluated are listed in Table I. The plot of log *P versus* the connectivity index is shown in Fig. 1. The log *P versus* the connectivity index for unsubstituted hydroxyureas at various concentrations of compound and different ratios of octanol to water is also shown in Fig. 1.

DISCUSSION

Drug action is known to be dependent on many events that take place after a drug is placed in a biological system, regardless of whether it is

[1] Courtesy of Miss B. Stearns, Squibb Institute of Medical Research.

Table II—Log *P* of Hydroxyurea at Different Concentrations in Octanol–Water

Dissolved Solute, g	Octanol–Water (1:1)	Octanol–Water (5:1)
0.1	−1.19	−0.42
0.5	−1.20	−0.57
1.0	−1.27	−0.74
2.0	−1.41	−1.52

an *in vitro* or *in vivo* system or whether the system is a human patient or an enzyme. Most of these events, such as membrane passage, adsorption or desorption to macromolecules, and attachment to the biochemical site of action with the resultant biological response, are dependent on the structure of the drug. Any interactions between or with the biological systems are, therefore, dependent on the atomic and molecular composition of the drug.

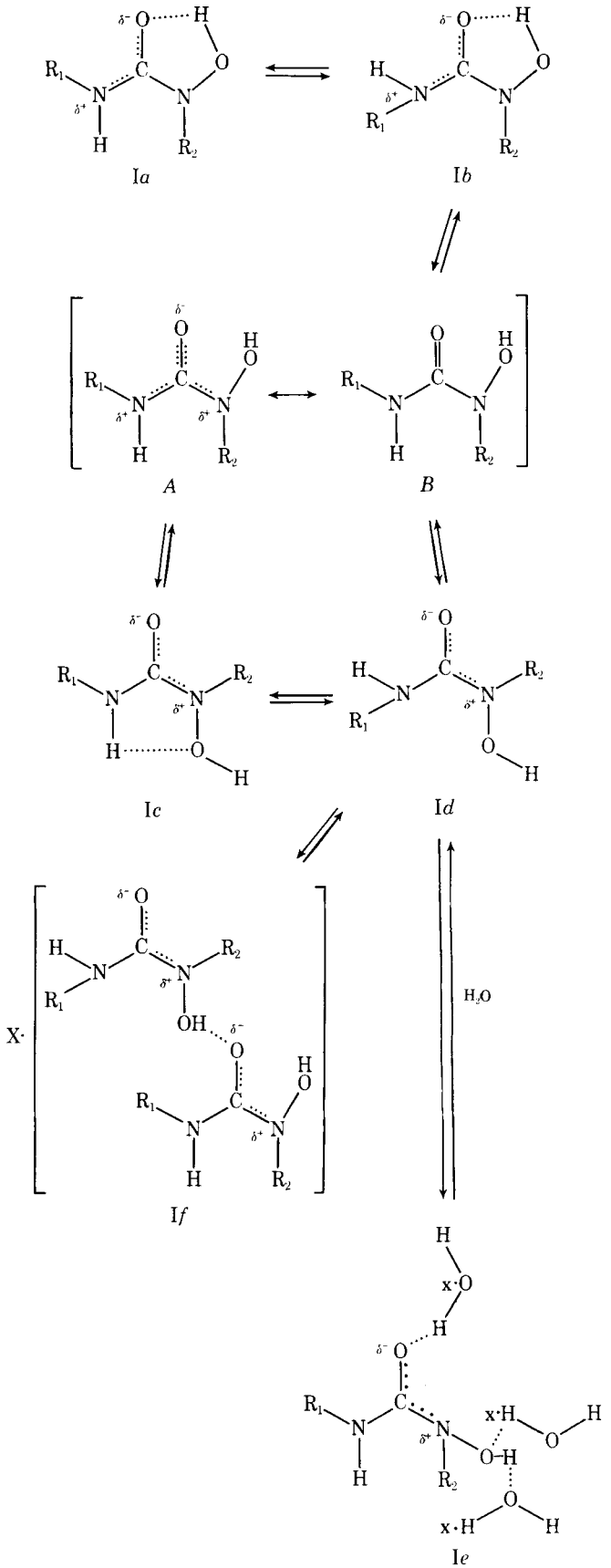
Because of this fundamental principle of structure dependence in biological action, many investigators attempted to correlate drug action with various basic properties of drugs such as their physical properties (polarization, log *P*, solubilities, *etc.*) or their absolute or potential molecular structure (quantum mechanical structural analysis, solvent dependent conformational analysis, *etc.*). While the utility of these methods may not be established, it is evident that the fundamental structure of a drug molecule is the basis for its action, although the processes may be so complex that facile study may be difficult. If these basic concepts are applied to a relatively simple drug entity such as the antileukemia agent hydroxyurea, some processes taking place in a biological system eventually leading to drug action (or inaction as the case may be) may be made clearer.

By applying some concepts developed by Hansch for the hydroxyurea series, it could have been postulated, after the original discovery of hydroxyurea activity (16), that molecular modification should produce a better anticancer agent. This hypothesis assumes that more lipophilic analogs should show more optimal drug action, since absorption can take place more readily and allow more drug to be available at the site of action through better drug distribution (17). The more lipophilic homologs usually pass through membranes with greater ease and often have greater biological action as long as solubility, bulk tolerance, or some other parameter does not diminish drug action (18). Since molecular modification has not succeeded in improving the antineoplastic properties of unsubstituted hydroxyurea (simple methyl substitution at the 1- or 3-nitrogen reduces action considerably), it becomes an academic question as to the reason for the dramatic change in activity with relatively minimal structural change.

Application of Hansch analysis for the prediction of log *P* values by the summing of fragments (*f* values) indicates that the predicted log *P* values are all more lipophilic than found experimentally except for hydroxyurea and the 1-methyl and the 3-*p*-chlorophenyl analogs (6). The greatest differences in predicted *versus* observed log *P* values in this series were with the 3-substituted straight chain analogs (ethyl, propyl, and butyl), all of which had Δ log *P* values of 0.70, while the bulky 3-substituted analogs (isopropyl and *tert*-butyl) had Δ log *P* values of 0.15 and 0.26, respectively. The hydroxyureas with log *P* values closest to those predicted were the 1-methyl, 1-ethyl, and 3-*p*-chlorophenyl analogs (Δ log *P* values of −0.14, 0.04, and −0.04, respectively). The predicted log *P* value for the parent hydroxyurea also was calculated to be appreciably different than that found experimentally (Δ log *P* of −0.91) (Table II), indicating that the hydroxyurea series did not behave as expected in the octanol–water partitioning system or that Hansch's method did not apply to a system such as the hydroxyureas except for certain analogs.

Since log *P* and most properties related to the physical characteristics of drugs are dependent on the molecular architecture of the molecule in question, it seems fitting to include those parameters that take into consideration the topological aspects of drug molecules. The method of molecular connectivity lends itself to the quantification of molecular architecture among the hydroxyureas. The molecular index of connectivity appears to be a quantification of structure that permits correlation with additive and constitutive properties of molecules. The connectivity index $^1\chi$ or $^1\chi^v$ of any molecule is the summed total of the reciprocal square root of the product of each bond in the molecule (excluding bonds to hydrogen), and the value obtained has been linearly correlated with polarizability, cavity surface area, local anesthetic action (9), water solubility and boiling points (19), the partition coefficient (7), and biological action (20) and parabolically related in certain drug classes to biological action (21).

When the molecular connectivity is calculated by the established



Scheme I—Conformational system of hydroxyureas depicted by canonical forms A and B, which allow interconversion to conformers Ia, Ib, and Ic, which possess internal hydrogen bonds, and to Id, which can interact with water (or solvent) to form solvate Ie or interact intermolecularly to form If.

procedure for the hydroxyureas under study and plotted against log P, two families of linearly related compounds are observed (Fig. 1) with hydroxyurea appearing between the two families (when hydroxyurea log P is determined in equal volumes of octanol–water). Compounds in the first group, composed of the 3-ethyl, 3-*n*-propyl, and 3-*n*-butyl analogs, are more hydrophilic than their counterparts in the second group, composed of the 1-ethyl, 3-isopropyl, and *tert*-butyl analogs, and include 1-methylhydroxyurea. Fundamental differences in the conformational equilibrium system of these hydroxyureas were identified previously by NMR (22) and IR (23) spectroscopy, and this behavior depended on the solvent.

Of the conformers possible in the hydroxyurea series (Scheme I), those stabilized by internal hydrogen bonding (Ia–Ic) are preferred by the members of the more lipophilic group (the 1-substituted and the 3-substituted bulky analogs). The more hydrophilic group appears to prefer the conformer that is not internally hydrogen bonded (Id) and thus can easily interact with solvent, water, or other hydroxyurea molecules (Ie and If). The partitioning characteristics of the hydroxyureas apparently depend on the ratio of the more lipophilic conformers (those internally hydrogen bonded) to those that can hydrogen bond to other species and thus are more hydrophilic. A compound of the first group would be expected to behave differently in a biophase in comparison to a compound of the second group because of the conformational preference. Although this behavior was apparent previously, it was not as markedly evident as when correlated with log P and molecular connectivity for the two classes of hydroxyureas.

In the plot of log P *versus* molecular connectivity, the parent drug hydroxyurea appears between the two other families of analogs when it is partitioned between equal volumes of octanol and water. This result most likely indicates that the conformational preference is not similar to that found in the other families of analogs but is probably a more equal distribution of possible conformers because of fast interconversions *via* rotations around nitrogen–carbonyl bonds. The conformational equilibria present in solution appear to differ for hydroxyurea and the 3-ethyl analog in the octanol phase when analyzed *via* the IR–X-ray crystal method of comparing solid and solution conformations (24), indicating that the conformational equilibria ratio for these two compounds is indeed different.

When log P for hydroxyurea is determined in a partitioning system of five volumes of octanol to one volume of water, the log P varies from −0.42 in a low concentration to −1.52 in a high concentration. This finding indicates that hydroxyurea behaves like a member of the more lipophilic class in low concentrations and approaches the behavior of the more hydrophilic class in high concentrations. This behavior can be rationalized according to Scheme I if it is considered that hydroxyurea can interact with: (*a*) itself (such as in conformers Ia–Ic), (*b*) lipophilic solvent when in all possible conformations, (*c*) water, or (*d*) other hydroxyurea molecules, as in conformer Id (to form solvate Ie and/or intermolecular bonded species If).

When the hydroxyurea concentration is low, the contribution of the conformer involved in intermolecular interactions is decreased, since this process is less likely to occur in lower hydroxyurea concentrations. The relative contribution of the internally bonded conformers increases, and the result is a greater partitioning into the more lipophilic octanol phase. At higher hydroxyurea concentrations, the likelihood of intermolecular interactions increases through the involvement of Id, increasing the overall contribution of this conformer in the equilibrium scheme. Interconversions through Id to species involving water or other hydroxyurea molecules may be favored rather than interconversion to conformers such as Ia–Ic, thus increasing the relative hydrophilicity of the molecular species involved. With the substituted hydroxyureas, concentration does not appear to play as significant a role in the equilibrium conformational scheme, possibly because the bulky substituents do not facilitate intermolecular interactions as easily as unsubstituted hydroxyurea.

In conclusion, the correlation of log P with valence molecular connectivity apparently delineates substituted hydroxyureas into two classes of compounds which differ in their conformational equilibria and relative lipophilicity–hydrophilicity. Unsubstituted hydroxyurea, on the other hand, can exist in different ratios of conformational equilibria, depending on the concentration in solution, but is not similar to either group of substituted analogs in equal volumes of octanol and water. In low concentrations, hydroxyurea appears more lipophilic in character and resembles the group of substituted analogs comprised of the 1- and 3-substituted bulky compounds. In higher concentrations, hydroxyurea becomes more hydrophilic and approaches the range of the other group of substituted analogs comprised of the 3-substituted straight chain compounds.

The unique behavior of hydroxyurea compared to the substituted analogs in regard to the different conformational equilibria present in an *in vitro* or *in vivo* biophase might be a factor in the low biological action of substituted hydroxyureas compared to the model compound. The concentration dependency observed in the partitioning of hydroxyurea between octanol and water may be clinically significant since a difference in an apparent log P of 1 unit or greater would appreciably change the absorption, membrane transport, and relative distribution potential (possibly to normal *versus* neoplastic tissue if more lipophilic) of the agent administered. This dependency on concentration in hydroxyurea warrants further study to determine if this property influences *in vitro* or *in vivo* antitumor activity by modifying the biodynamics involved in biological action.

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ACKNOWLEDGMENTS

Presented in part at the Fourth Annual Southcentral Medicinal Chemistry Meeting in Miniature (MALTO), Houston, Tex., May 1977, sponsored by Texas Southern College of Pharmacy.

The author thanks Miss Barbara Stearns of the Squibb Institute of Medical Research for the hydroxyurea, 1-methylhydroxyurea, and 1-ethylhydroxyurea.