Molecular Connectivity and Retention Indexes

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Abstract □ The molecular connectivity indexes, χ, of some alcohols, ethers, esters, and ketones did not correlate very well with their retention volumes in some GLC systems. It was concluded that the utility of χ for the prediction of biological activities could prove rather limited.

Keyphrases □ Molecular connectivity indexes—attempted correlation with GLC retention indexes of various alcohols, ethers, esters, and ketones □ GLC retention indexes—attempted correlation with molecular connectivity indexes of various alcohols, ethers, esters, and ketones □ Topological indexes—molecular connectivity indexes correlated with GLC retention indexes of various alcohols, ethers, esters, and ketones

Recently, the molecular connectivity index, χ, was used to predict several parameters related to biological activities of drugs (1). One of these parameters was log P, the partition coefficient between octanol and water, which is one of the three principal parameters in the Hansch equations (2) that are used to predict (or rationalize) biological activities of drugs. The authors concluded (1) that direct correlation of molecular connectivity to biological activity is possible.

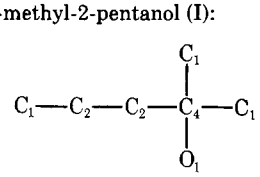
If the biological activity of a substance is to be predicted from χ, then this property must be correlated with the magnitude of the diverse physicochemical interactions the substance is subjected to from resorption to receptor. These interactions are often very specific in nature. Even log P is a rather complex quantity, since it is determined by interactions with two different phases. Therefore, if χ is to have some general value, it should be sufficiently correlated with interactions of diverse natures.

To have a more precise idea of the potential value of χ, it was of interest to study interactions with some simpler systems. Since large compilations of GLC data exist (3) and since GLC retention is the result of a relatively simple equilibrium between a liquid phase and a gas, the correlation of χ with retention indexes (R.I.) was investigated.

EXPERIMENTAL

The connectivity index, χ, is calculated in the following way (1). The chemical formula is written down in the skeletal form, and each atom is assigned a number corresponding to the number of atoms attached. Hydrogen atoms are suppressed. For carbon atoms, the number equals 1, 2, 3, or 4. An ether oxygen is assigned the number 2; the N-atoms of the amines RNH and R_2N are assigned the numbers 2 and 3, respectively. The product of the numbers associated with the two atoms of one bond is then found. The sum of the reciprocal square root of these products is calculated to give a number, called the connectivity index.

For example, for 2-methyl-2-pentanol (I):



I

$$\chi = \frac{1}{\sqrt{2}} + \frac{1}{\sqrt{4}} + \frac{1}{\sqrt{8}} + \frac{1}{\sqrt{4}} + \frac{1}{\sqrt{4}} + \frac{1}{\sqrt{4}} = 3.061 \quad \text{(Eq. 1)}$$

Retention indexes are the logarithms of the adjusted retention volume (log V_R') relative to those of normal paraffins. Correlations between χ and the retention index were obtained for four stationary phases, namely the apolar squalane, the polar diethylene glycol succinate and polyethylene glycol 300, and one rather special phase[1] (II), which has unique properties (4, 5) and interacts strongly with ketones.

Table I—Correlation Coefficients between the Retention Index and χ

Compound Family	Squalane	Diethylene Glycol Succinate	Polyethylene Glycol 300	II
Alcohols	0.95	0.76	0.78	0.92
Esters	0.99	0.91	0.96	0.98
Ketones	0.99	0.94	0.96	0.98
Ethers	0.97	0.71	0.82	0.71
All compounds	0.91	0.55	0.56	0.73

Four groups of substances were studied. The first included methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 3-pentanol, 1-hexanol, 2-hexanol, 3-hexanol, 1-heptanol, 1-octanol, 2-methyl-2-butanol, 3-methyl-2-butanol, 2,2-dimethyl-1-propanol, 2-methyl-2-pentanol, 3-methyl-2-pentanol, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 3-methyl-3-pentanol, 2,4-dimethyl-3-pentanol, 3-ethyl-3-pentanol, and cyclohexanol.

The second group included acetone, 2-butanone, 2-pentanone, 3-pentanone, 3-methyl-2-butanone, 2-hexanone, 3-hexanone, 3-methyl-2-pentanone, 4-methyl-2-pentanone, 2-heptanone, 3-heptanone, 2,4-dimethyl-3-pentanone, 2-nonanone, and 5-nonanone.

The third group consisted of ether, methyl butyl ether, methyl propyl ether, isobutyl methyl ether, *tert*-butyl methyl ether, dipropyl ether, and isopropyl propyl ether. And the fourth group included ethyl formate, propyl formate, methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, *sec*-butyl acetate, methyl propanoate, ethyl propanoate, methyl butanoate, ethyl butanoate, and pentyl acetate.

The χ values for these substances were taken from Murray *et al.* (1), and the retention index values were taken from the data of McReynolds (3).

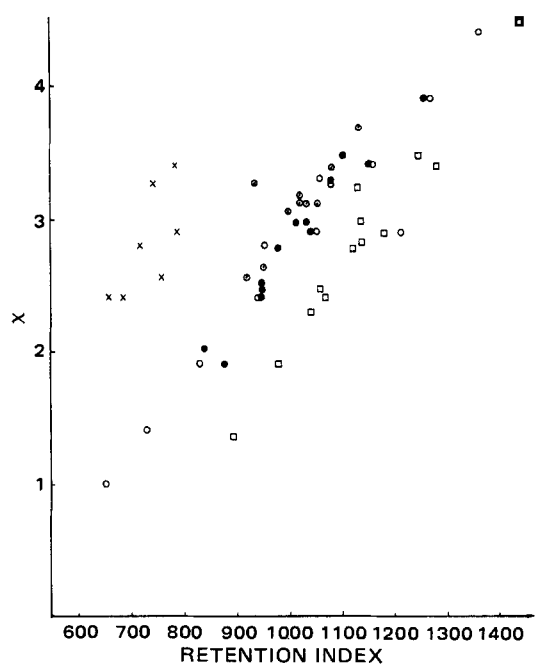


Figure 1—*Plot of* χ versus *retention indexes obtained using II as the stationary phase. Key:* ○, *straight chain alcohols;* ⊙, *branched chain alcohols;* ●, *esters;* □, *ketones; and* ×, *ethers.*

[1] Zonyl E7.

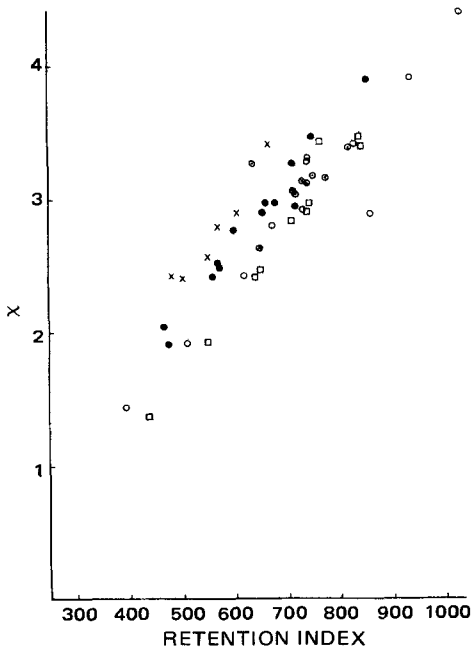


Figure 2—*Plot of* χ versus *retention indexes obtained using squalane as the stationary phase. Key:* ○, *straight chain alcohols;* ⊙, *branched chain alcohols;* ●, *esters;* □, *ketones; and* ×, *ethers.*

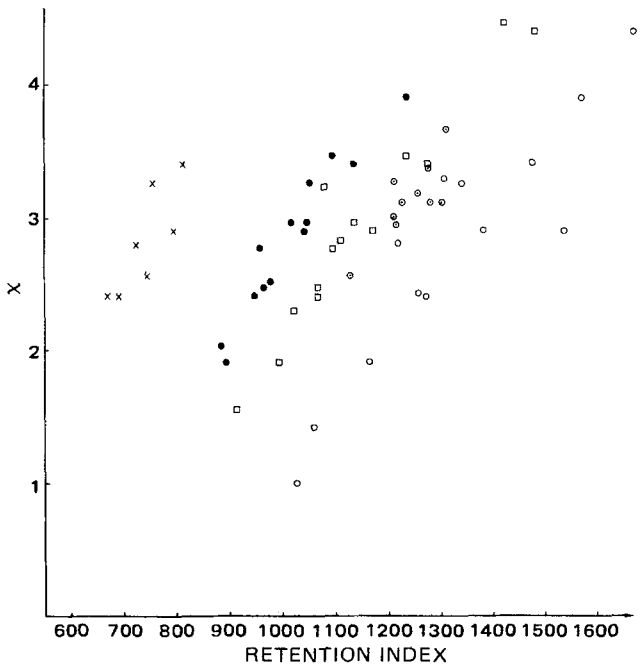


Figure 4—*Plot of* χ versus *retention indexes obtained using polyethylene glycol 300 as the stationary phase. Key:* ○, *straight chain alcohols;* ⊙, *branched chain alcohols;* ●, *esters;* □, *ketones; and* ×, *ethers.*

DISCUSSION

The correlation coefficients are given in Table I, and the results are shown in Figs. 1–4. While some correlations are good, many correlation coefficients are lower than 0.976, the lowest correlation found between χ and log P for a class of compounds (the ether family) by Murray *et al.* (1).

Of particular interest is the behavior of the alcohols. The retention index values of the straight chain alcohols are perfectly correlated with χ. This result was, of course, to be expected since the retention index values of these substances plotted as a function of the number of carbon atoms fall on a straight line and since χ is also a linear function of the number of carbon atoms. On the two polar phases, however, the branched chain alcohols do not fall on this straight line; cyclohexanol is a point by itself, apparently unrelated to the other alcohols.

The ethers, esters, and ketones fall on other lines rather distant from that of the straight chain alcohols. This result leads to correlation coefficients for all compounds on the two polar phases of 0.5–0.6.

The interactions on the two polar phases may be unrepresentative of what happens in biological systems; but if the molecular connectivity is a fundamental property, as advocated by Murray *et al.* (1), a better correlation should be expected. From the results obtained with the two polar phases, one can anticipate that rather bad results will be obtained, at least in some cases, for the prediction of biological activities.

The correlation on squalane is better but less than would be expected from the performance of χ for the parameters studied previously (1). Squalane is a hydrocarbon, and its interactions resemble those of octanol or the lipid phase for which the latter is representative in the Hansch equations. Here again, the clear segregation of the four families of compounds results in a correlation coefficient of 0.91. This segregation points out that the primary defect of χ is that the role of functional groups such as hydroxyl and carbonyl is not sufficiently taken into account. This fact is demonstrated by the results with II. Where, on other phases, the ketone line is found before (at a lower retention index) or at about the same retention index as the alcohol line, it lags rather considerably behind on II. As stated before, II is known to interact with ketones.

The object of this report is not to say that χ is useless. It is a topological quantity which, as shown conclusively (1), allows prediction or rationalization of some interesting parameters. However, it may be of more limited use than can be expected from the previous conclusion (1) and, in any case, the results obtained should be used with caution, particularly when there is reason to believe that functional groups play a determining role in the biological activity.

Kier and coworkers altered the way of calculating the molecular connectivity index[2]. They developed extended connectivity functions, which encode additional information about multiple paths in a molecule. They also developed nonempirical self-consistent parameters, which can be used to treat heteroatoms of interest in biology. The improved connectivity calculations might allow a better prediction of retention indexes than was obtained here with the simple χ parameter.

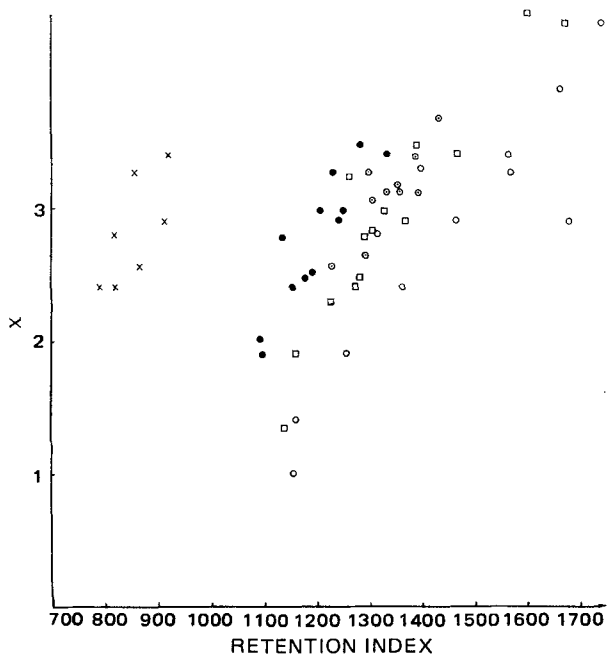


Figure 3—*Plot of* χ versus *retention indexes obtained using diethylene glycol succinate as the stationary phase. Key:* ○, *straight chain alcohols;* ⊙, *branched chain alcohols;* ●, *esters;* □, *ketones; and* ×, *ethers.*

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Fluorometric Determination of Methyldopa in Biological Fluids

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Abstract □ A fluorometric method for the analysis of methyldopa, based on the formation of a fluorophore after oxidation and rearrangement, is described. The drug is isolated from biological fluids by adsorption on alumina and elution with an organic solvent. Fluorescence is linear from 0.1 to 1.5 μg of methyldopa/ml. The assay has a lower limit of sensitivity of 100 ng/ml and is suitable for pharmacokinetic studies following therapeutic doses in animals and humans.

Keyphrases □ Methyldopa—fluorometric analysis, biological fluids □ Fluorometry—analysis, methyldopa in biological fluids □ Antihypertensive agents—methyldopa, fluorometric analysis in biological fluids

Methyldopa is a widely used antihypertensive agent with sedative and aromatic L-aminodecarboxylase-inhibiting properties (1–7). Methods to analyze the drug in pharmaceutical preparations and biological fluids have utilized visible spectrophotometry (8, 9), GLC (10), high-pressure liquid chromatography (11), and fluorometry (12, 13). In addition, tracer methodology has been used for metabolic and disposition studies (14, 15).

Fluorometric methods have been primarily based on the production of a fluorescent indole derivative (lutin) by oxidation and subsequent rearrangement. This technique has also been applied to estimate catecholamines and related compounds (16). Existing fluorometric methods appear to be insufficiently sensitive for the determination of drug levels in biological fluids.

This paper describes an improved analytical method in which methyldopa is adsorbed on alumina, eluted with an organic solvent, and then oxidized to form the fluorophore, dihydroxyindole. The establishment of optimal conditions for maximal fluorescence resulted in an increased sensitivity for estimating methyldopa in biological fluids. The method has provided increased sensitivity, consistency, and reproducibility.

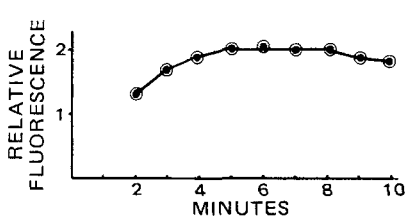

Figure 1—*Stability of methyldopa oxidation product.*

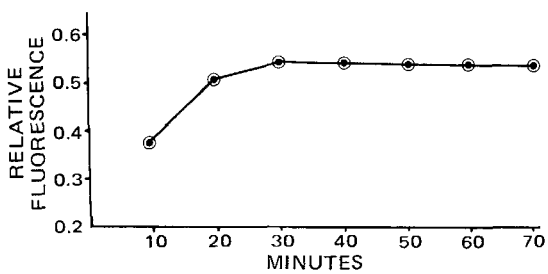

Figure 2—*Stability of fluorophore of methyldopa following oxidation and rearrangement.*

EXPERIMENTAL

Apparatus—All fluorescence measurements were made using a spectrophotofluorometer[1]. Microphotometer sensitivity was set at 30 with multiplier positions ranging from 0.01 to 0.1.

Reagents—All chemicals and solvents were analytical reagent grades, and freshly distilled water was used. All glassware was rinsed with 10% nitric acid and distilled water. Alumina[2] was reactivated by refluxing in 2 *N* HCl, washing with distilled water until the pH was 3.5–4, and drying at 250–300°. Methyldopa was used as received[3].

The phosphate buffer was prepared by adding 0.1 *M* dibasic sodium phosphate containing 0.1% edetate sodium to 0.1 *M* monobasic sodium phosphate containing 0.1% edetate sodium.

Analytical Procedure—Blood samples were collected in heparinized tubes containing 15 mg of sodium metabisulfite. The blood was centrifuged immediately, and the plasma was separated and frozen until assayed. Aliquots of biological fluids (0.1–1.0 ml) were placed in screw-capped test tubes, and 4–5 ml of 0.4 *N* perchloric acid was added. After shaking for 5 min, the precipitated protein was separated by centrifugation.

A specific volume (1–5 ml) of the supernate was transferred to a screw-capped test tube containing 0.5 g of activated alumina and 0.25 g of edetate sodium. The mixture was adjusted to pH 8.5–8.6 with 1 *N* sodium carbonate with constant stirring, and shaking was continued for an additional 5 min. The alumina was allowed to settle, and then the supernate was aspirated and discarded. The alumina was washed twice with 5 ml of distilled water, and the washings were discarded. The adsorbed methyldopa was eluted by vigorously shaking the washed alumina for 15 min with 6 ml of acetone–formic acid (85:15).

The alumina suspension was centrifuged, and 5.0 ml of the supernate was transferred to a test tube and evaporated under a nitrogen stream at 40°. The dried residue was dissolved in 1 ml of pH 6.5 phosphate buffer. Then 0.1 ml of ethanol, 0.05 ml of 0.2% zinc sulfate, and 0.05 ml of 0.1%

[1] Aminco-Bowman, American Instrument Co.
[2] Woelm Neutral Activity Grade 1.
[3] Courtesy of Merck Sharp & Dohme Laboratories.