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Physicochemical and Topological Correlates of the Enzymatic Acetyltransfer Reaction

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Abstract □ The relative potencies of a series of substituted anilines as acetyl acceptors in the enzymatic *N*-acetylation reaction have been correlated using physicochemical substituent constants (π, σ^-), molecular connectivity indices ($^1\chi$, $^1\chi^v$), and newly formulated information–theoretic topological indices (IC, SIC). Results indicate a predominant role of the topological steric parameters in determining the rates of the *N*-acetyltransferase reaction.

Keyphrases □ *p*-Nitroaniline—determination of the *N*-acetylation reaction, topological indices □ Topological indices—information–theoretic, rate determination of the *N*-acetylation reaction, comparison with physicochemical constants and molecular connectivity indices, substituted anilines □ *N*-Acetylation reaction—of substituted anilines, enzymatic acetyltransferase, rate determination using information–theoretic topological indices

The biochemical acetyl transfer reaction is important not only for normal physiological processes, but also in the extramicrosomal metabolism of therapeutically active compounds like isoniazid (1, 2), *p*-aminosalicylic acid (3), sulfonamides (4, 5), and anticancer drugs–*viz.*, 6-aminonicotinamide (4). Acetyltransferase is capable of catalyzing the transfer of the acetyl moiety from acetyl CoA (CoASAc) to aliphatic and aromatic amines as well as the reversible transfer of an acetyl group between different aromatic amines (6, 7). Therefore, one of the probable ways of elucidating the molecular basis of this reaction might arise from the study of acetyl group transfer rates from a particular acetylated amine (donor) to other variously substituted amines (acceptor) that vary in their physicochemical and geometrical characteristics in a well-defined manner.

Jacobson (6) studied the rates of acetyl transfer from *p*-(*p*-acetylaminophenylazo)benzenesulfonate to a series of substituted anilines in the presence of purified pigeon liver acetyltransferase. The electronegativity of the substituent(s) was conjectured to have an overwhelming role on the rates of the reaction. This notion gained support from the quantum chemical studies of Perault and Pullman (8) where the electronic charge on the amine nitrogen (ϵ) of the acceptor was shown to parallel the acetylation rate. Further studies by Hansch *et al.* (9) using substituent constants derived from physical organic model systems showed that a biparametric relationship using hydrophobic (π) and electronic (σ^- or ϵ) parameters could adequately correlate the biological data.

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Table I—Partitioning of *p*-Nitroaniline

Partition Class	Coordinate	Number of Atoms in the Partitioned Class[a]	Probability ($p_i = n_i/n$)
I	1^4	4	4/16
II	1^3	2	2/16
III	2^5	2	2/16
IV	$1^4 2^2 2^2$	1	1/16
V	$1^1 1^1 1^4$	1	1/16
VI	$1^1 1^4 2^4$	4	4/16
VII	$1^4 1^5 2^4$	1	1/16
VIII	$1^3 1^4 2^4$	1	1/16

[a] Total number of atoms in the molecule = 16.

One of the important outcomes of the Hansch analysis in this area was that the steric factor is insignificant in enzymatic acetyl transfer process, a conclusion drawn from a study of six compounds. Jacobson (6) studied the *N*-acetylation of 10 substituted anilines. Although the physicochemical substituent constants for the remaining four compounds were not available then (9), some of them can be derived today. Moreover, in recent years, a number of information–theoretic (10–14) and molecular connectivity-type (15–20) topological indices have been derived and found to be of use in biological correlations. While valence molecular connectivity ($^m\chi^v$) indices are excellent electronic parameters (21), simple connectivity indices ($^m\chi$) and information–theoretic indices derived from the topological neighborhood of vortices (atoms) in the molecular graph (structure) are steric parameters that encode information regarding the topological shape of the molecule (11–15, 22). Although the action of nonspecific bioactive molecules is mainly guided by their hydrophobicity, the *in vivo* and *in vitro* activity of specific biologically active agents, *i.e.*, molecules which act by virtue of being recognized by an enzyme or a physiological receptor, is highly dependent on stereoelectronic makeup associated with the molecular architecture (23, 24).

Hence it was of interest to examine whether steric parameters have any role in the mechanism of action of *N*-acetyltransferase. To this end, correlations of the rates of enzymatic acetylation of amines with the physicochemical substituent constants (π, σ^-), molecular connectivity indices ($^1\chi$, $^1\chi^v$), as well as information–theoretic topological indices (11), *i.e.*, information content (IC) and structural information content (SIC), have been attempted. Also, a comparative study of the role of physicochemical *versus* theoretical parameters in the correlation of biological data has been undertaken using multiparametric regression equations.

EXPERIMENTAL

Calculation of the Hydrophobic Constant (π)—The hydrophobic parameter for the various substituents in the aniline derivatives are taken from Hansch and Leo (25). In cases where more than one substituent is present, the combined lipophilic effect is calculated by the addition of the individual contributions of the groups with respect to lipophilicity.

Calculation of the Electronic Parameter (σ^-)—The σ^- values for the various groups in the aniline system are taken from Hansch *et al.* (9, 25), Hoefnagel *et al.* (26), and Zeng (27). Since the simple additivity rule is obeyed with respect to electronic parameters of the Hammett type from a single system (28), the electronic effects (σ^-) of more than one substituent in the ring is taken to be the sum of their individual contributions in the aniline system.

Calculation of Molecular Connectivity ($^1\chi$)—To each atom of the hydrogen-suppressed graph a δ value is assigned corresponding to the number of nonhydrogen atoms bonded to it. No consideration is made to the class of atom or type of bond. A connectivity value for a bond C_k (connecting atoms i and j) is computed as follows (15):

$$C_k = (\delta_i \delta_j)^{-1/2} \qquad \text{(Eq. 1)}$$

And $^1\chi$ is calculated as the sum of all connectivity terms:

$$^1\chi = \sum_k C_k \qquad \text{(Eq. 2)}$$

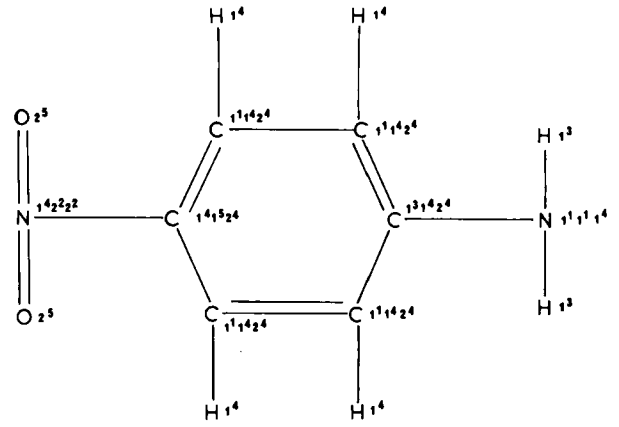

Figure 1—*Coordinate-attached structure of* p-*nitroaniline.*

Calculation of Valence Molecular Connectivity ($^1\chi^v$)—To each atom of the hydrogen-depleted skeleton a δ^v value is assigned as follows (15, 21):

$$\delta^v = Z_i^v - h_i \qquad \text{(Eq. 3)}$$

where Z_i^v is the number of valence electrons and h_i is the number of hydrogen atoms attached to the particular atom. Thereafter, $^1\chi^v$ is calculated in a similar manner as $^1\chi$ by substituting δ^v for δ in Eq. 1.

Calculation of Information Content (IC) and Structural Information Content (SIC) from the Molecular Graph—In this graph–theoretic formalism, the total (nonhydrogen-suppressed) molecular graph is considered to define the various topological indices. The method is sufficiently general to include the linear graphs as well as multigraphs. If G is a molecular graph with vertex set $X(G)$ and $A_i (i = 1, 2, \ldots, k)$ is a partition of $X(G)$, then a probability scheme is given by:

$$\begin{pmatrix} A_1, A_2, \ldots, A_k \\ p_1, p_2, \ldots, p_k \end{pmatrix}$$

where $p_i = n_i/n$, and n_i and n are the cardinalities of A_i and $X(G)$, respectively. Then, the information content (IC) of the graph G with respect to this mode of partition of $X(G)$ is given by Shannon's formula (29):

$$IC(X(G)) = -\sum_k p_i \log_2 p_i \text{ bits} \qquad \text{(Eq. 4)}$$

The logarithm is taken at a basis 2 to measure the information in bits. By defining a first-order topological neighborhood and an equivalence relation on the vertex set $X(G)$ of a molecular graph, Sarkar *et al.* (10) computed the first-order topological information content of various molecular graphs. Subsequently Basak *et al.* (30) defined another information–theoretic topological index, structural information content (SIC):

$$SIC = IC/\log_2 n \qquad \text{(Eq. 5)}$$

Let us take a member of the series of anilines, *p*-nitroaniline (Fig. 1), to exemplify the partitioning of atoms into disjoint classes (Table I) and also the calculation of IC and SIC. In this graph–theoretic treatment, which is patterned after the work of Sarkar *et al.* (10), all four hydrogen atoms attached to the aromatic ring lie in the same disjoint subset. This partitioing scheme differs from that proposed by Kier (14) where chemical intuition is also used in the decomposition of the vertex set of the molecular graph into disjoint subsets.

RESULTS AND DISCUSSION

Table II shows the π and σ^- values of the substituents, $^1\chi$ and $^1\chi^v$ indices from the hydrogen-suppressed molecular graphs, IC and SIC indices

Table II—Biological Properties and Molecular Descriptors for Aniline Derivatives

Compound	Relative Rate (A_x)	σ^-	π	$^1\chi$	$^1\chi^v$	IC (bits)	SIC
Aniline	0.70	0.00	0.00	3.3938	2.1994	2.0060	0.5269
p-Me-Aniline	1.00	−0.17	0.48	3.7877	2.6100	2.3432	0.5733
p-Cl-Aniline	1.09	0.23	0.93	3.7877	2.7120	2.0138	0.5289
p-Br-Aniline	1.12	0.23	1.13	3.7877	3.1021	2.0185	0.5301
p-NO_2-Aniline	0.34	1.27	0.50	4.6984	2.6988	2.7500[a]	0.6875[b]
Sulfanilamide	0.18	0.91	−1.16	4.9900	3.1110	2.8795	0.6778
p-NH_2-Salicylic acid	0.15	0.38	−0.99	5.1091	2.9280	3.3083	0.7934
p-NH_2-Benzoic acid	0.03	0.77	−0.32	4.6984	2.7878	3.0286	0.7409
Sulfanilic acid	0.01	0.48	−4.76	4.9980	3.0422	3.0588	0.7335

[a] $IC = 2 \times 4/16 \log_2(16/4) + 2 \times 2/16 \log_2(16/2) + 4 \times 1/16 \log_2(16/1) = 2.7500$ bits. [b] $SIC = IC/\log_2(16) = 0.6875$.

from the total chemical graphs, and the relative rates of enzymatic acetylation (A_x) for a series of substituted anilines; the experimental data was taken from the work of Jacobson (6). Since our approach was to have a comparative study between the physicochemical and theoretical descriptors in the correlation of acetylation rates, *p*-aminohippuric acid was not considered for correlation because the σ^- value for the *para* substituent was not available.

Table III shows the correlation matrix (31) for A_x and the six molecular descriptors under study. High correlations are observed between the variables A_x, IC, SIC,and $^1\chi$. The parameters $^1\chi^v$, π, and σ^- are poorly correlated with A_x and the other three descriptors. Linear correlation results of each of the six descriptors with A_x are:

$$A_x = -0.616\,(^1\chi) + 3.20 \qquad \text{(Eq. 6)}$$
$$n = 9 \quad r = 0.88 \quad s = 0.24 \quad F_{1,7} = 23\ (p < 0.002)$$

$$A_x = -0.473\,(^1\chi^v) + 1.84 \qquad \text{(Eq. 7)}$$
$$n = 9 \quad r = 0.30 \quad s = 0.48 \quad F_{1,7} = 0.68\ (p < 0.439)$$

$$A_x = -0.830\,(\text{IC}) + 2.67 \qquad \text{(Eq. 8)}$$
$$n = 9 \quad r = 0.91 \quad s = 0.20 \quad F_{1,7} = 35\ (p < 0.001)$$

$$A_x = -4.07\,(\text{SIC}) + 3.13 \qquad \text{(Eq. 9)}$$
$$n = 9 \quad r = 0.92 \quad s = 0.20 \quad F_{1,7} = 36\ (p < 0.001)$$

$$A_x = 0.181\,(\pi) + 0.598 \qquad \text{(Eq. 10)}$$
$$n = 9 \quad r = 0.70 \quad s = 0.36 \quad F_{1,7} = 6.7\ (p < 0.036)$$

$$A_x = -0.648\,(\sigma^-) + 0.807 \qquad \text{(Eq. 11)}$$
$$n = 9 \quad r = 0.64 \quad s = 0.38 \quad F_{1,7} = 4.8\ (p < 0.065)$$

Here n is the number of data points, r the correlation coefficient, s the standard deviation, and F is the F-ratio between the variances of the observed and calculated values.

Equations 6–11 account for 76.8, 8.7, 83.4, 83.7, 48.7, and 40.6% of the variance (r^2) in A_x, respectively. It is clear from these results that steric (topological) parameters like $^1\chi$, IC, and SIC have a very important role in determining the rates of enzymatic acetylation as compared with the hydrophobic (π) or electronic (σ^- or $^1\chi^v$) parameters. The same trend is obtained with the parabolic correlations of A_x with $^1\chi$, $^1\chi^v$, IC, SIC, and π:

$$A_x = 0.462\,(\pi) + 0.0761\,(\pi)^2 + 0.494 \qquad \text{(Eq. 12)}$$
$$n = 9 \quad r = 0.87 \quad s = 0.26 \quad F_{2,6} = 9.8\ (p < 0.013)$$

$$A_x = 2.97\,(^1\chi) - 0.418\,(^1\chi)^2 - 4.34 \qquad \text{(Eq. 13)}$$
$$n = 9 \quad r = 0.90 \quad s = 0.23 \quad F_{2,6} = 12\ (p < 0.007)$$

$$A_x = -0.782\,(^1\chi^v) + 0.0574\,(^1\chi^v)^2 + 2.25 \qquad \text{(Eq. 14)}$$
$$n = 9 \quad r = 0.30 \quad s = 0.51 \quad F_{2,6} = 0.29\ (p < 0.759)$$

Table III—Correlation Matrix

	A_x	π	$^1\chi$	$^1\chi^v$	IC	SIC	σ^-
A_x	1.00						
π	0.70	1.00					
$^1\chi$	−0.88	−0.62	1.00				
$^1\chi^v$	−0.30	−0.36	0.63	1.00			
IC	−0.91	−0.63	0.95	0.46	1.00		
SIC	−0.92	−0.60	0.94	0.43	0.99	1.00	
σ^-	−0.64	−0.16	0.69	−0.37	0.55	0.53	1.00

$$A_x = -1.73\,(\text{IC}) + 0.174\,(\text{IC})^2 + 3.79 \qquad \text{(Eq. 15)}$$
$$n = 9 \quad r = 0.92 \quad s = 0.224 \quad F_{2,6} = 16\ (p < 0.004)$$

$$A_x = -17.60\,(\text{SIC}) + 10.5\,(\text{SIC})^2 + 7.39 \qquad \text{(Eq. 16)}$$
$$n = 9 \quad r = 0.93 \quad s = 0.20 \quad F_{2,6} = 18\ (p < 0.003)$$

Of the nine compounds in Table II, the last seven are homogeneous in the sense that each of them has an electron-withdrawing substituent in the position *para* to the amino group. Therefore, it is expected that steric fit may better correlate the biological data for such a homogeneous group of compounds. Regression equations (Eqs. 17–20) show this to be the case:

$$A_x = -6.67\,(^1\chi) + 0.666\,(^1\chi)^2 + 16.8 \qquad \text{(Eq. 17)}$$
$$n = 7 \quad r = 0.98 \quad s = 0.13 \quad F_{2,4} = 41\ (p < 0.002)$$

$$A_x = -4.49\,(\text{IC}) + 0.695\,(\text{IC})^2 + 7.35 \qquad \text{(Eq. 18)}$$
$$n = 7 \quad r = 0.99 \quad s = 0.09 \quad F_{2,4} = 93\ (p < 0.001)$$

$$A_x = -28.5\,(\text{SIC}) + 18.6\,(\text{SIC})^2 + 11.0 \qquad \text{(Eq. 19)}$$
$$n = 7 \quad r = 0.99 \quad s = 0.10 \quad F_{2,4} = 69\ (p < 0.001)$$

$$A_x = 0.451\,(\pi) + 0.0781\,(\pi)^2 + 0.411 \qquad \text{(Eq. 20)}$$
$$n = 7 \quad r = 0.90 \quad s = 0.25 \quad F_{2,4} = 9.0\ (p < 0.033)$$

The addition of the hydrophobic parameter, π, to Eqs. 17 and 18 made only marginal improvement in the correlation coefficient.

It is evident from the above that topological indices reveal a predominant role of steric factor in determining the rate of the enzymatic *N*-acetyltransferase reaction, although Hansch *et al.* (9) could assign no role of the steric descriptor with a smaller subset of these compounds. With nine compounds a topological parameter such as $^1\chi$, IC, or SIC emerged to be the single variable best suited for correlation of this type of bioactive agent. The significant relationships developed above may be utilized in the rational design of substrates for *N*-acetyltransferase.

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Pharmacokinetics and Anesthetic Potency of a Thiopental Isomer

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Received March 9, 1982, from the *Departments of Anesthesia and Medicine (Clinical Pharmacology), Stanford University School of Medicine, Stanford, CA 94305* and the *Palo Alto Veterans Administration Hospital, Palo Alto, CA 94304.* Accepted for publication July 29, 1982. [†] Deceased

Abstract □ In developing a high-performance liquid chromatographic assay for thiopental [5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid], a thiopental isomer [5-ethyl-5-(1-ethylpropyl)-2-thiobarbituric acid] was found. This isomer occurs (6–7%) in supposedly pure thiopental and in the commercially available thiopental sodium administered to patients for induction of anesthesia. A similar type of isomer also occurs in pentobarbital, the oxybarbiturate analogue of thiopental. Because the disposition and anesthetic potency of the isomer is unknown, its pharmacokinetic properties were determined in humans and its anesthetic potency in mice. In five surgical patients, the terminal elimination half-life, clearance, and volume of distribution at steady state of the isomer were not statistically different from those of thiopental. In mice, the isomer proved to be as effective as thiopental for induction of anesthesia. The LD_{50} and sleep time at one-half the LD_{50} did not statistically differ between the two compounds in mice. The close structural similarity of thiopental and the isomer results in similar pharmacokinetic and anesthetic properties. It does not appear critical that the isomer be separated from thiopental in subsequent pharmacological research.

Keyphrases □ Thiopental—isomer determination in serum by high-performance liquid chromatography, pharmacokinetics in humans, anesthetic potency in mice □ Pharmacokinetics—of a thiopental isomer in humans, comparison with thiopental □ Anesthetic agents—potency of a thiopental isomer in mice, comparison with thiopental

Previous high-performance liquid chromatographic (HPLC) assays for thiopental apparently lacked the chromatographic resolution to separate thiopental from any side-chain isomer (1–4). This paper describes an HPLC assay that separates thiopental, 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid, from this isomeric material, 5-ethyl-5-(1-ethylpropyl)-2-thiobarbituric acid (see Fig. 1). The isomer was found both in standard thiopental obtained from the manufacturer and the commercially available thiopental sodium used for the induction of anesthesia in patients. It apparently is formed during the manufacture of thiopental[1]. The presence of a similar isomer has been described for pentobarbital [5-ethyl-5-(1-methylbutyl) barbituric acid], the oxybarbiturate analogue of thiopental (5).

EXPERIMENTAL

Apparatus and Reagents—A liquid chromatograph[2] was equipped with a variable-wavelength detector[3] and column heater. The phosphoric acid, monobasic potassium phosphate, sodium hydroxide, and sodium carbonate were certified ACS grade[4]; anhydrous ethyl ether was reagent grade[5]; acetonitrile was HPLC grade[6]. The sodium salt of thiopental[7] and the thiopental isomer[8] were used for preparation of the standard curves. The thiopental contained 9.0% of the isomer, based on peak heights from a sample separated on the HPLC assay. The isomer did not contain thiopental; only a single peak was seen when the isomer was chromatographed on the HPLC assay. Thiamylal acid[9] was used as an internal standard for measurement of thiopental and the isomer.

Thiopental Protein Precipitation and Chromatography—The HPLC assay described by Kabra *et al.* (6) was modified. For thiopental concentrations >200 ng/ml, an equal volume of acetonitrile containing the internal standard (2.5 or 25 μg/ml) was added to human serum (200 μl) and mixed on a vortex mixer. Following two sequential centrifugations, the acetonitrile supernatant was injected into the chromatograph.

[1] Personal communication, Abbott Laboratories, North Chicago, Ill.
[2] Model 5020, Varian, Palo Alto, Calif.
[3] Model UV-50, Varian, Palo Alto, Calif.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] J. T. Baker Chemical Co., Phillipsburg, N.J.
[6] Distilled in glass; Burdick & Jackson Laboratories, Muskegon, Mich.
[7] Lot 845-7283, Abbott Laboratories, North Chicago, Ill.
[8] Abbott 13750, Lot No. 16-859-AX, Abbott Laboratories, North Chicago, Ill.
[9] Lot 7274 x 24-6, Parke-Davis & Co., Detroit, Mich.

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