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Structure-Metabolism Relationships: Steric Effects and the Enzymatic Hydrolysis of Carboxylic Esters

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Abstract: After a brief review of a number of issues related to the enzymatic hydrolysis of carboxylic esters, such as interspecies variability, mechanism, stereospecificity, and activation energy, and after an overview of relevant aspects related to the quantitative modeling of steric effects, the results of a recently developed quantitative structure-metabolism relationship model are discussed. They were obtained for *in vitro* human blood enzymatic hydrolysis of noncongener esters by introduction of the inaccessible solid angle as a novel measure of steric hindrance.

INTRODUCTION

Because of the important role played by enzymatic hydrolysis in the pharmacokinetic behavior of ester- or amide-containing therapeutic agents, there has been a long-standing interest in the characterization of the corresponding enzymes [1-7] and in the development of adequate quantitative structure-metabolism relationships (QSMR) [8-12]. Unfortunately, progress in these fields was considerably hindered by the broad and overlapping substrate specificity of these enzymes. Recently, a three-dimensional structure-based method that uses the inaccessible solid angle as a novel steric parameter to estimate human blood *in vitro* enzymatic hydrolysis rates was developed on the basis of experimental data obtained from different series of noncongener ester-containing drugs, Fig. (**1**) [13]. The model is of interest because it represents the first QSMR model for enzymatic hydrolysis not limited to congener series, and because it seems to be in general agreement with the currently accepted mechanism of enzymatic hydrolysis. Also, it is of special interest for those working in the fields of prodrug [14-16] and soft drug design [17, 18], since these strategies mainly rely on enzymatic hydrolysis for drug activation and deactivation, respectively.

ENZYMATIC HYDROLYSIS

The hydrolysis of a variety of ester-containing chemicals into the respective free acids is very efficiently catalyzed by carboxylic ester hydrolases (EC 3.1.1). These ubiquitons enzymes exhibit broad and overlapping substrate specificity toward esters and amides, and the same substrate is often hydrolyzed by more than one enzyme. Consequently, their classification is difficult and still is in a confused state, despite the important roles that carboxylesterase (EC 3.1.1.1) and/or other carboxylic ester hydrolases, such as arylesterase (EC 3.1.1.2) and cholinesterase (EC 3.1.1.8), play in the metabolism of many xenobiotics [1-7]. The emergence of a novel classification system developed on the basis of adequate sequence information, which is starting to accumulate, might provide a possible solution to this problem [7]. Interestingly, in many cases, the physiological role of carboxylesterases still remains somewhat unclear [6]. Humans have been shown to express carboxylesterase in the liver, plasma, small intestine, brain, stomach, colon, macrophage, and monocytes [7].

Interspecies Variability

It is well known that esterase activity varies considerably between species [1-7, 19]. For aliphatic esters, the rate of hydrolysis usually decreases in the rat > rabbit > dog > human order, but there might be considerable variability [20-22]. Rodents (rats, guinea pigs) tend to metabolize ester-containing drugs much faster than humans. For example, *in vitro* hydrolytic half-lives ($t_{1/2}$) measured in rat blood were often found orders of magnitude lower than those measured in human blood as illustrated, for example, by clevidipine (0.6 min vs 5.8 min) [22], esmolol (2.3 min vs 26–27 min) [20, 23], or remifentanil (0.5 min vs 37 min) [24]. However, flestolol, which contains an aromatic ester, showed an opposite trend ($t_{1/2}$ of 54 min in rat blood vs 1 min in human blood) [25]. In a number of cases, aliphatic esterases presumably absent from dog and human plasma, but present in rat and guinea pig plasma, were suspected as causes of the observed large differences [23]. The rank order of compounds tends to be similar in different biological systems, but even this cannot be considered a general rule [26, 27].

In addition to the usual problems related to the extrapolation [28, 29] of animal test results to humans, this strong interspecies variability further complicates early drug evaluations for ester-containing compounds. Furthermore, a recent investigation of the metabolism of flestolol and other esters found polymorphic rates of ester hydrolysis in New Zealand white rabbit blood and cornea [25]. About 30% of the animals studied (n = 86) were found as "slow" metabolizing (*in vitro* blood half-lives of $t_{1/2}$ = 17 min), and about 70% were found as "fast" metabolizing ($t_{1/2}$ < 1 min).

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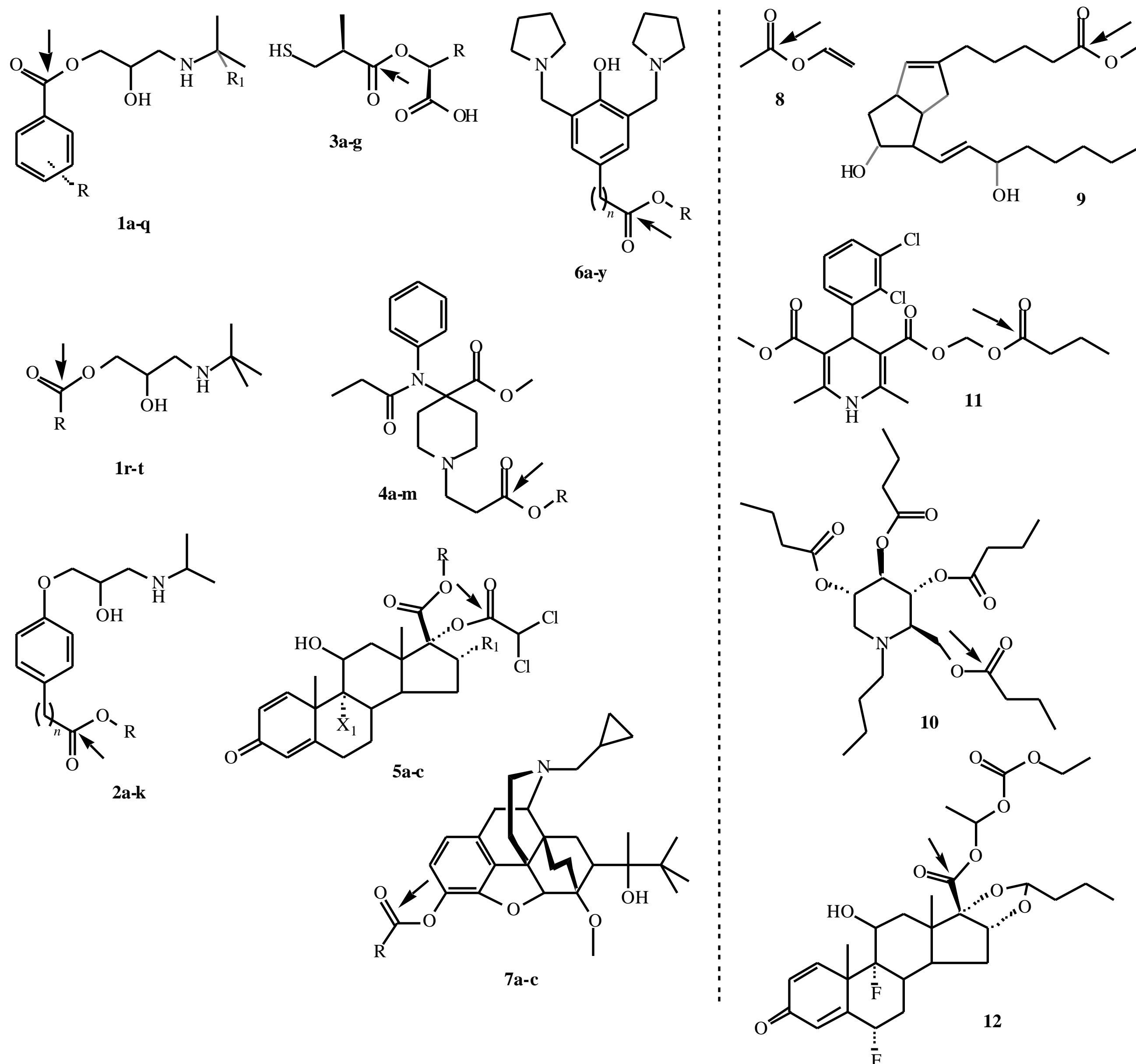

Fig. (1) Structures used in the present QSMR study. Experimental hydrolysis data from the series **1**-**7** were used to fit the multiple linear regression model (eq. 5, Fig. (**6**)), and data from compounds **8**-**12** were used to test the predictive power (Fig. (**7**)). Arrows indicate the sites of the enzymatic hydrolysis considered.

Interestingly, no such bimodal distribution of esterase activity was found in blood from rats, dogs, and humans or in the aqueous humor and iris-ciliary body complex of rabbits [25].

On the other hand, the differential distribution of these enzymes can be exploited to provide metabolism-based selectivity. Malathion (**13**), one of the most generally useful loco-systemic compounds, provides an elegant example for the selectivity that can be achieved for pesticides by exploiting the differences in the enzymatic constitution of vertebrates and insects, Fig. (**2**). Malathion is detoxified through a variety of metabolic pathways, one of the most prominent one being the hydrolysis of one of its two ethyl carboxylester groups. The carboxylesterase that hydrolyzes and thereby detoxifies malathion is widely distributed in mammals, but only sporadically in insects, where in some rare cases is responsible for insecticide resistance (see [30, 31] and references therein). In the meantime, insects seem to posses a very active oxidative enzyme system that transforms malathion (**13**) into malaoxon (**14**), a much more active cholinesterase inhibitor. Probably, all insects and all vertebrates possess both an esterase and an NADPH-dependent oxidase system, but the balance of action of these two systems varies from one organism to another and provides this selectivity of action. It is, therefore, quite likely that by incorporating the possibility of similar mechanisms into the structure of other insecticides from the parathion family, which tend to have unacceptably high mammalian toxicities and are activated by a similar

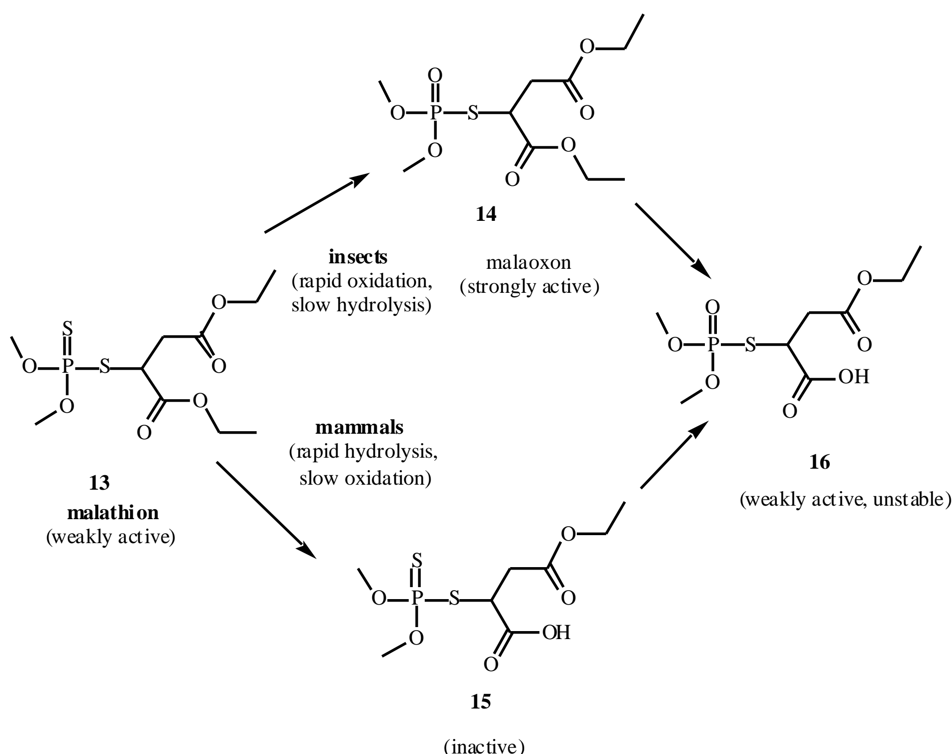

Fig. (2). Malathion (**13**), its oxidative activation to malaoxon (**14**), a much more active cholinesterase inhibitor, and its deactivation by carboxylesterases. The carboxylesterase that hydrolyses and thereby detoxifies malathion is widely distributed in mammals, but only sporadically in insects thus accounting for the selective toxicity to insects and the relative safety to mammals [30, 31].

oxidation, safer and more selective chemicals can be designed.

Mechanism – Present Knowledge

Recently, a mechanism was proposed for hydrolysis by carboxylesterase (see [7] and references therein) on the basis of conserved motifs in various carboxylesterases and following other, similar mechanisms [32, 33]. It was suggested that this mechanism involves Ser[203], Glu[335], and His[448] as a catalytic triad, in which low-barrier hydrogen bonds facilitate a general base mechanism for the acylation of Ser[203], together with Gly[123]-Gly[124] as part of an oxyanion hole, in which weak hydrogen bonds stabilize the tetrahedral adduct, Fig. (**3**). Sequences required for hydrolytic capability at the catalytic triad seem to be highly conserved in carboxylesterase, acetylcholinesterase, butyrylcholinesterase, and cholesterol esterase [7].

Stereoselectivity and Activation Energy

Stereoselectivity is an important aspect of enzyme-catalyzed reactions, but only relatively limited data are available on the stereoselectivity of enzymatic hydrolysis within the pharmaceutical field. Nevertheless, evidence of stereoselective hydrolysis has been found in various media for a number of cases, such as ester prodrugs of oxazepam [34, 35], propranolol [36-39], or ibuprofen [40]. In the last case, *R*:*S* rate ratios as high as 50 were reported. As a more extreme example, the behaviorally inactive (+)-cocaine was found to hydrolyze at least 1,000 times faster in baboon plasma than (–)-cocaine, the naturally occurring enantiomer of cocaine [41]. For cocaine hydrolysis, considerable stereoselectivity was also found in rat hepatocytes [42]. As data on the mechanism of various enzymatic hydrolysis are becoming to accumulate, one can hope that elucidation of the rationale behind the enantioselectivity of certain hydrolytic enzymes may also become possible (see, e.g., [43] and references therein).

The temperature-dependence of the rate of hydrolysis in human blood has been studied for esmolol [44] and clevidipine [22]. As expected, it was found that as media (blood) temperature decreases, the rate (k) decreases and, hence, the half-life ($t_{1/2} = \ln 2 / k$) increases. In both cases, but especially in that of clevidipine, good linearity was observed between the logarithm of the rate (or the half-life) and the inverse of the absolute temperature ($1/T$) as required by an Arrhenius-type equation, $k = A \exp(-E_a/RT)$ [45]. The corresponding slopes indicate apparent activation energies E_a of 55.5 kJ/mol (n = 4, r^2 = 0.9586) and 76.6 kJ/mol (n = 3, r^2 = 0.9998) for esmolol and clevidipine, respectively. This appears to be in reasonable agreement, for example, with the activation energy of approximately 75 kJ/mol found for lactose hydrolysis by recombinant beta-glycosidases [46] or that of 43.5 kJ/mol found for anandamide hydrolysis by human brain fatty-acid amide hydrolase [47]. It might also be of interest to note that the activation energies found for the chemical hydrolysis of liposomal phosphatidylcholine under acidic conditions (pH 4.0) were also mostly in the 60-70

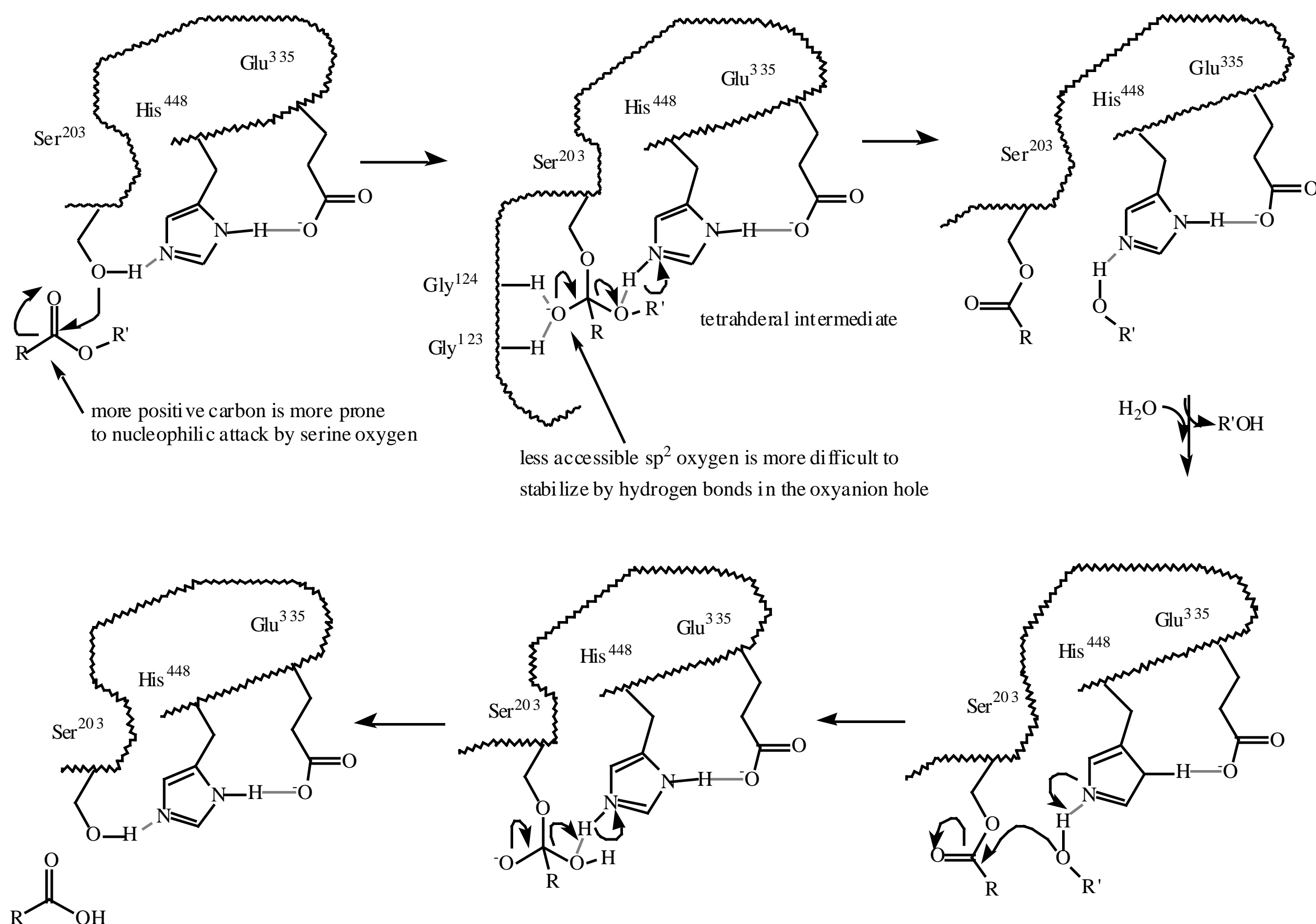

Fig. (3). Illustration of the mechanism proposed for hydrolysis by carboxylesterases on the basis of analogy with other, similar mechanisms and a study of highly conserved motifs [7]. The mechanism involves Ser^{203}, Glu^{335}, and His^{448} as a catalytic triad and Gly^{123}–Gly^{124} as part of an oxyanion hole. The QSMR model represented by eq. 5 agrees well with such a mechanism as indicated here.

kJ/mol range [48]. Extrapolation of the above-mentioned *in vitro* enzymatic hydrolysis results for ester-containing drugs such as esmolol and clevidipine to *in vivo* situations has to be done carefully. Nevertheless, the data suggest that a reduction in body temperature from 37°C to around 30°C, which is routinely done during cardiac surgery, may approximately double the half-life of such drugs [22]. Indeed, pharmacokinetic studies in hypothermic and normothermic patients with remifentanil and clevidipine showed prolonged half-life and reduced clearance at lower temperatures [22, 49].

Structure–Metabolism Relationships

The effect of structure on enzymatic half-life has been investigated on a large variety of pharmaceuticals, usually members of some prodrug or soft drug series [9-12, 20, 23, 24, 26, 27, 40, 50-74]. Nevertheless, establishing useful quantitative structure-metabolism relationships of general validity that go beyond the obvious "increasing steric hindrance, such as that produced by branched substituents, increases half-life" proved unusually difficult here. The significance of the ability to introduce rigorously measurable, quantitative aspects into structure-activity, structure-property, or similar type of relationships is often overlooked despite this being probably the most important contribution of such QSAR-type studies to the advancement of medicinal chemistry and drug design [75-80]. QSMR attempts within selected individual ester-containing series were made by Charton [8], by Altomare and co-workers [10], and by Testa and co-workers [9, 11, 12]. Such studies may provide general guidelines, but they are essentially useless for other, noncongener series. Furthermore, the unavoidable structural similarity present in such congener series can make many of the available parameters strongly intercorrelated making it very difficult to clearly identify the real source of variability.

Recently, a more general relationship was identified [13] on the basis of human blood *in vitro* metabolism data of more than 80 compounds belonging to seven different classes: two -blocker series with ultrashort duration of action **1a-t** [26] and **2a-k** [20, 23, 63, 67], ultra-short-acting angiotensin converting enzyme inhibitors **3a-g** [68], opioid analgetics **4a-m** [24], soft corticosteroids **5a-c** [81], short-acting antiarrhythmic agents **6a-y** [64, 65], and buprenorphine prodrugs **7a-c** [69], Fig. (**1**). *In vitro* human blood data was used because it represented the data of interest available in the largest number over the widest range

of structures under comparable experimental conditions. The predictive power of the model was tested on three separate ester-containing drugs with completely unrelated structures in the original publication: vinyl acetate (**8**) [82], isocarbacyclin methyl ester (TEI-9090, **9**) [21], and glycovir (**10**) [83]). Two further structures, clevidipine (**11**) [22] and itrocinonide (**12**) [84], will be added here.

STERIC EFFECTS: QUANTITATIVE MODELS

Reflecting the important role played by steric effects in chemical or enzymatic reactions, numerous methods have been developed for their quantification; they have been reviewed in several publications [78-80, 85, 86]. However, steric effects are inherently difficult to characterize as they strongly depend on the 3D structures involved, and these can vary considerably due to intramolecular motions and intermolecular interactions. In addition, structures for drug binding sites are rarely known with adequate accuracy.

Steric Parameters – Overview

Recognition of the rate-influencing role played by the size of substituents, introduction of the *steric effect* term, and development of a first quantitative measure of steric effects date back to the late 1800's [87-92]. Nevertheless, the first successful quantitative steric parameter, Taft's steric constant E_s, was introduced only in the 1950s [93, 94] following an earlier proposition of Ingold [95]. This steric constant was defined based on the change in the rate constant k of the acid catalyzed hydrolysis produced by a substituent X in X-CH_2COOR type esters:

$$E_s = \log\left(\frac{k_x}{k_H}\right)_A \qquad (1)$$

Following a suggestion by Hansch and co-workers, in order to reference the E_s scale to hydrogen, published values are usually rescaled by subtraction of 1.24, a value obtained for the hydrolysis of HCOOR [78]. Despite a long suspected contamination with electronic effects, this experimentally derived constant was the only available steric parameter that proved successful for a long time. A number of variations have been introduced. For example, Dubois and co-workers introduced a new set of E'_s values by using a single reference reaction, the acid-catalyzed esterification of carboxylic acids in methanol at 40°C [96]. Hancock introduced E_s^c values corrected for the number of -hydrogen atoms, n_H, in an attempt to account for possible hyperconjugation effects [97]:

$$E_s^c = E_s + 0.306(n_H - 3) \qquad (2)$$

Fujita and co-workers expressed the E_s^c values of -$CR^1R^2R^3$ type substituents as the weighted sum of the individual E_s^c values of R^1, R^2, and R^3 to overcome problems with steric parameters of unsymmetrical substituents [98].

One of the first quantitative steric parameters introduced on the basis of geometric considerations was that of Amoore

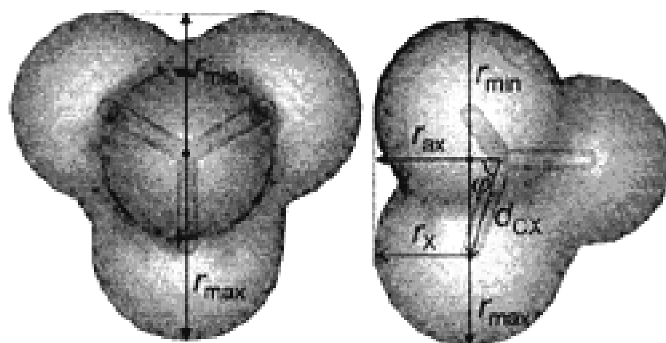

Fig. (**4**) Definition of the linear steric parameters (r_{ax}, r_{max}, r_{min}) used by Charton to introduce his steric parameter, = r_{min} − 1.20, illustrated for the case of a CCl_3 substituent, which is bonded to a C atom and is represented by transparent van der Waals surfaces. Two perpendicular views are shown: one along the C–C axis (left) and another one perpendicular to this and to one of the C–C–Cl planes (right). The STERIMOL parameters introduced by Verloop and co-workers have more general, but very similar definitions.

and co-workers, 1/(1 + $_{avg}$), obtained by comparing the similarity of the planar outlines (silhouettes) of entire molecules (so-called shadow matching) [99, 100]. The first general substituent steric parameter defined solely on the basis of geometric considerations was introduced by Charton [101, 102]. For symmetrical substituents such as CX_3, he defined three different van der Waals radii: one along the group axis ($r_{ax} = d_{CX} \cos \;\; + r_X$) and two in perpendicular directions to the group axis ($r_{max} = d_{CX} \sin \;\; + r_X$, $r_{min} = d_{CX} \cos \;\; \cos 60° + r_X$), Fig. (**4**). Noticing that they, and especially r_{min}, tend to correlate well with E_s, he defined a new steric parameter that was re-scaled using the radius of the hydrogen atom r_H:

$$= r_{min} - r_H = r_{min} - 1.20 \qquad (3)$$

However, generalization of this geometrical definition was not straightforward for unsymmetrical substituents. Consequently, correlations with experimental log $(k_X)_A$ values were used to calculate $_{eff}$ values for such substituents. Taft's E_s and Charton's have indeed been shown to be strongly correlated [78]. In fact, by extending an idea of Charton, Kutter and Hansch derived a linear regression equation connecting E_s and the average radii of several substituents and then used this equation to estimate E_s values for substituents that had no experimentally determined value [103]. Bowden and Young used a steric substituent constant R, calculated using molecular models as the distance from the atom to which the substituent is bonded to the periphery of the van der Waals radius of the substituent [104]. A set of more complex directional parameters, the so-called STERIMOL parameters L, B_1–B_4, B_5, were introduced based on quite similar geometric considerations by Verloop and co-workers [105, 106]. They were defined as the length of the substituent along the axis of substitution (L) and four width parameters perpendicular to this axis and forming 90° angles with each other ($B_1 < B_2 < B_3 < B_4$). Later, B_5, a width parameter having the largest

value independent of the angle relative to B_1, was also introduced. The B_1 parameter showed high correlation with Charton's constant, which has a very similar definition, and also with Taft's E_s [86]. A substituent steric effect index ( ) based on the molecular graph and introduced by Kier [107] that also correlates well with E_s and a steric parameter (Y) introduced by Nádasdi and Medzihradszky [108] for amino acid side chains based on their fractional van der Waals volume also should be mentioned here.

Such linear measures proved useful descriptors in a number of cases, but since steric effects result from 3D structures, measures of the spatial angle around the reaction center should give a less arbitrary and more accurate description of steric accessibility. Within this context, Tolman introduced cone angles obtained from CPK models to characterize steric effects of phosphorus ligands [109, 110]. Somewhat later, Immirzi and Musco suggested using a generalized non-circular cone with a corresponding solid angle as determined from X-ray structural data [111]. The steady exponential development in computational power made it possible to calculate more rigorous measures of steric accessibility, and in 1984 Seeman and co-workers used the accessible solid angle evaluated with a Monte Carlo sampling as a measure of the geometric accessibility factor for nitrogens in pyridines [112]. In an attempt to generalize this concept, Sakakibara, Hirota, and co-workers defined a steric substituent constant $_s$, which basically represents the portion of the total solid angle that is hindered by the substituent considered [113-117]. They used molecular mechanics optimized structures and a population-weighted average value obtained from different possible conformers to account for conformational effects. A reasonable correlation was obtained between $_s$ and E_s (r = 0.887) that improved considerably when only alkyl substituents having no heteroatoms were considered (r = 0.953), suggesting that electronic contamination in E_s for heteroatom-containing substituents is a likely cause for deviation. White, Taverner, and co-workers also attempted the quantification of steric effects by the use of solid angles while developing a different method of calculation [118-120]. Brown, White, and co-workers introduced and developed a different computational measurement of steric effects, the molecular-mechanics-based ligand repulsive energy, E_R [121, 122].

Inaccessible Solid Angle

The solid angle can be considered a 3D generalization of the 2D (planar) angle concept. Mathematically, the solid angle subtended at a point O by an arbitrary surface S is defined by the surface integral

$$= {}_{s} \frac{\mathbf{r} \cdot d\mathbf{S}}{r^3} \qquad (4)$$

where $\mathbf{r}$ is the position vector of the element of surface $d\mathbf{S}$ with respect to O [123]. For a spherical surface centered at O, $= S/r^2$ (compare with the definition of the planar angle subtended by a circular arc of length l, $= l/r$). is measured in steradians, and the steric angle subtended by a full sphere is 4 steradians (compare with the 2 radians angle subtended by a full circle) [124]. The area of the shadow of the surface S projected on a circumscribing sphere by a light placed in the center O is a good visual measure of the solid angle , since, after all, represents 4 times the ratio of this area to that of the whole sphere, Fig. (**5**).

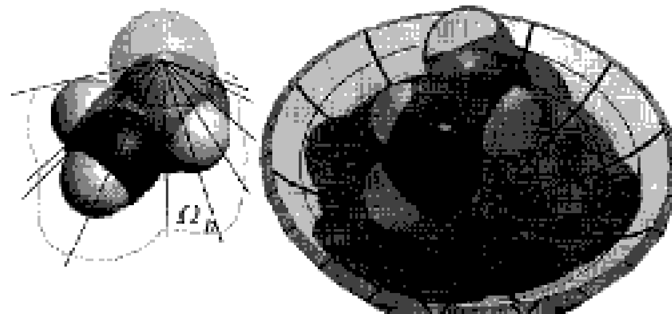

Fig. (5). Two illustrations of the inaccessible solid angle $_h$ hindered around a selected atom by a 1-chloro-2-hydroxyethyl substituent (chlorine in the back). The atom for which the hindered, inaccessible solid angle is illustrated is at the top of both figures represented as a transparent sphere with a small, white center (left) and a transparent "glass" sphere (right), respectively. The area of the shadow projected by the light placed in the center of the selected atom on a circumscribing sphere is proportional with $_h$. For transparency, this atom was made of "glass", which also causes a distortion of the light-rays passing through it (top of the right-hand figure).

On the basis of spatial considerations, the accessible solid angle probably represents the most rigorous structure-related measure of the probability that a reagent molecule can access the reaction center in a given configuration. Hence, the inaccessible solid angle $_h$, the solid angle at which access to a reaction center is hindered by substituents, should be a good measure of steric hindrance. Nevertheless, such measures of steric hindrance have been applied only in a few chemical studies [112-117, 125] and have been completely overlooked until now in pharmaceutical studies. The inaccessible surface area of an atom, or more precisely the ratio of inaccessible to total surface area, represents a measure very similar to $_h$, but $_h$ can also account for the hindering effect of structurally more distant atoms.

$_h$ is, by definition, independent of electrical or transport-related effects, and computer models allow reasonably fast and sufficiently accurate evaluations for any center of interest within any molecular structure. By using such a calculated total value, one can account for the effect of all the substituents and avoid the problems related to the additivity or nonadditivity of the different substituent contributions. Also, if reactivity at the center is not isotropic, a directionally weighted form can be easily computed by using, for example, a $\cos^2$ type function that corresponds to the electron density of a p orbital. The only major problem that has to be adequately treated is the conformation-dependency of $_h$. The value of $_h$ as defined here may vary considerably for flexible molecules; therefore, meaningful values can be obtained only if some energetic considerations are included in the computational procedure. Values used here were computed from AM1 (Austin Model 1, [126]) optimized structures after a careful conformational sampling to select the energetically favored conformers with the sterically less hindered ester positions.

Using the van der Waals surface of the molecules, the total inaccessible solid angle around any atom can be evaluated relatively easily with simple numerical techniques. The van der Waals surface or volume concept already proved successful in many applications, and even if in an exact quantum chemical description the electron cloud has no well-defined boundary surface, the empirical success of this concept gives a good starting point for simple computational models. Furthermore, since Bader and co-workers [127] have shown that contours of constant electronic density (0.001-0.002 electron/bohr^3) that contain over 96% of the total electronic charge gave good representations of the molecular van der Waals surfaces or of the smoother contact surfaces, their use is justifiable even by more rigorous physicochemical standards.

The total inaccessible steric angle $_h$ used here was computed with a numerical algorithm implemented and integrated within our previous computer software package [13, 128]. Directional sampling points were obtained with a regular sampling grid using spherical coordinates. For every atom for which $_h$ had to be computed, any direction that somewhere crossed the van der Waals surface of another atom in the molecule was considered as hindered. The final $_h$ values used here simply represent the percentage of hindered direction points: $_h = 100 \times N_{hindered}/N_{total}$ because such a relative value is more descriptive than an absolute value expressed in steradians.

QSMR FOR ENZYMATIC HYDROLYSIS

$_h$-Based Model

In general agreement with previous results, our quantitative study also found steric effects as having the most important influence on the rate of enzymatic hydrolysis as measured by the *in vitro* half-life in human blood. Lipophilicity, as measured by the calculated log octanol-water partition coefficient (log $P_{o/w}$), and some of the electronic parameters, such as the charge on the carbonyl C ($q_{C=}$), also proved informative, but to a much lesser degree.

As expected, half-lives were found to increase with increasing steric hindrance around the ester moiety. However, an important novelty was the finding that the rate of metabolism as measured by log $t_{1/2}$ seems to be more strongly correlated with the steric hindrance of the carbonyl sp^2 oxygen as measured by $_h^{O=}$ (r^2 = 0.58, with all data included n = 79) than with that of the carbonyl sp^2 carbon as measured by $_h^{C=}$ (r^2 = 0.29). Since the correlation with $_h^{O=}$ is much better than with $_h^{C=}$ and is also considerably better than that for the overall ester group (r^2 = 0.42 for $_h^{COO} = {}_h^{O=} + {}_h^{C=} + {}_h^{O}$), we suggested that this provides evidence for the important, possibly even rate-determining role played by hydrogen bonding at this oxygen atom in the mechanism of this reaction. We also suggested that the H-bonds formed within the oxyanion hole not only help stabilize the tetrahedral adduct in the second step, but they already play an important role in the first step of the mechanism presented in Fig. (**3**) by a partial proton transfer that makes the sp^2 carbon more susceptible to the nucleophilic attack of the catalytic triad.

It has to be mentioned that for a set of 40 structurally diverse simple methyl esters, $_h^{C=}$ actually gave a better correlation with Taft's E_s steric constant than $_h^{O=}$ (r^2 = 0.73 vs 0.59). This suggests one possible explanation for the long-known observation (e.g., [51]) that chemical hydrolysis rates (apparently more closely related to the steric hindrance of the reaction center, the carbonyl C) tend to afford just very low correlations with enzymatic hydrolysis rates (apparently more closely related to the steric hindrance of the carbonyl O). It also suggests that the ability of this solid angle approach to compute steric hindrance at different atoms might be useful in distinguishing between different mechanisms.

It also should be mentioned that the inaccessible solid angles computed here for this set of 40 methyl esters did not give very good correlations with Taft's steric constant. Multiple halosubstituted compounds gave the largest deviations, suggesting again that electronic effect may indeed be still contaminating E_s. The correlation obtained with our calculated values on these 40 data were about the same quality as those obtained with the $_s$ steric substituent as defined by Sakakibara, Hirota, and co-workers and taken from their publication (r^2 = 0.78) [116].

The obtained final equation (eq. 5) used to estimate log $t_{1/2}$ values also includes the AM1-calculated charge on the carbonyl carbon ($q_{C=}$) and a calculated log octanol-water partition coefficient (QLogP) [129-132] as parameters and accounts for 80% of the variability in the log half-lives of 67 compounds, Fig. (**6**). All its parameters are statistically relevant ($p < 0.01$), but the present form was obtained after omission of twelve outliers. However, eight out of the twelve compounds omitted from the final correlation have very short half-lives that are difficult to determine and the corresponding experimental error might be considerable especially on a logarithmic scale.

$$\log t_{1/2} = -3.805(\pm 1.412) + 0.172(\pm 0.012)\ {}_h^{O=} - 10.146(\pm 3.439)\ q_{C=} + 0.112(\pm 0.044)\ \text{QLogP}$$
$$n = 67,\ r = 0.899,\ \ = 0.356,\ F = 88.1 \qquad (5)$$

We found no need to include second or higher order terms. Because the included parameters are not strongly

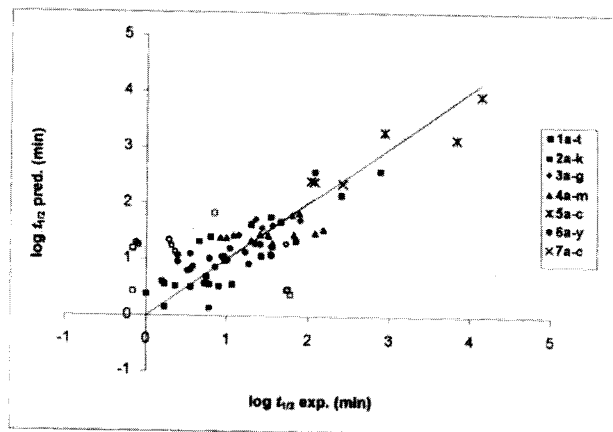

Fig. (**6**) Predicted vs experimental log half-lives for data used to fit the present QSMR model. Compounds omitted from the final correlation were denoted with an open symbol.

intercorrelated, the model is sufficiently robust from a statistical point of view. All of the parameters in this equation are calculable from the molecular structure; hence, it is possible to obtain half-life estimates even for compounds that have not been synthesized yet.

Equation 5 accounts for about 80% of the variance in log $t_{1/2}$ (Fig. (**6**)) (58% if all data are included), and it is interesting to note that most of the unexplained variance remains within the different series and not between the different series. The correlations obtained are not very good, but considering that we have biological data on seven different drug series from a number of different investigators, they can be considered quite informative. In addition, since most likely a number of different enzymes are involved in the hydrolysis of these compounds, one can hardly expect any general description at this level to give a significantly better overall fit. It has to be mentioned, however, that within some of the series a number of compounds were found not to be metabolized in any significant amount and the corresponding (large) $t_{1/2}$ were not reported at all. For most of them, our model fails to predict a half-life significantly larger than those of their structurally similar analogues. It is possible that some of their structural features hinder the fit into the active site of the metabolizing enzyme(s), but no such features were obvious.

Another important aspect is that this $_h$-based QSMR equation (eq. 5) agrees very well with the mechanism summarized earlier for enzymatic hydrolysis, Fig. (**3**). A more positive carbon ($q_{C=}$) is more prone to the nucleophilic attack by the serine oxygen, and a less accessible carbonyl oxygen ($_h^{O=}$) is more difficult to stabilize by hydrogen bonds in the oxyanion hole.

Predictive Power

The predictive power of the present model was originally tested by estimating the half-lives of three separate drugs with completely unrelated structures and experimental data available: vinyl acetate (**8**), isocarbacyclin methyl ester (**9**), and glycovir (**10**). Here, two further structures were added: clevidipine (**11**) and itrocinonide (**12**). The agreement between predicted and experimental half-lives is still reasonable as shown in Fig. (**7**).

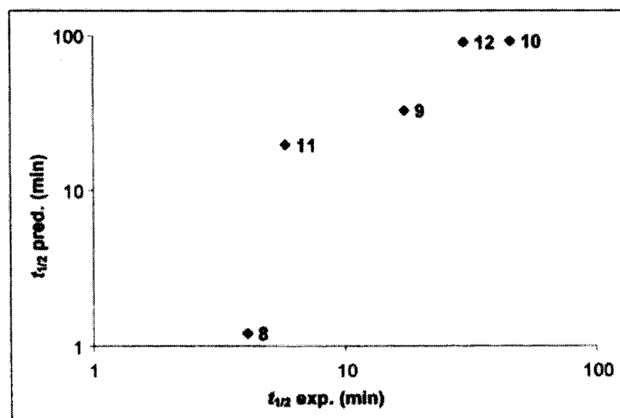

Fig. (**7**) Predicted (eq. 5) vs observed in vitro human blood hydrolysis half-lives on a logarithmic scale for five compounds (vinyl acetate **8**, isocarbacyclin methyl ester **9**, glycovir **10**, clevidipine **11**, and itrocinonide **12**) used to test the predictive power of the present model.

It is unrealistic to expect accurate predictions of hydrolytic half-lives for arbitrary structures, but the present method should prove useful in distinguishing among compounds whose hydrolysis is fast, medium, or slow on the basis of chemical structure alone. Two important warnings have to be mentioned here. First, considering that eq. 5 is based on logarithmic half-lives, has a standard deviation of = 0.36, and that differences of up to two standard deviations are usually not considered outliers (especially in a predictive setting), differences of up to five-times ($10^{2\times0.36}$ = 5.25) in the predicted value of the half-lives themselves are entirely possible. Second, because $_h$ is considerably conformation-sensitive, and because a small change in its value can cause large variations in the calculated $t_{1/2}$, careful conformational sampling is required to find the less hindered energetically favorable conformation before any estimates are made.

Obviously, eq. 5 cannot account for any specific effect. For example, insertion of a heteroatom substituent (in particular, sulfur) in the or position relative to the carbonyl was noted to dramatically increase the rate of enzymatic hydrolysis [70], and similar observations were also made for soft ACE inhibitors [68] or -blockers [67]. For such S atoms, a possible role in stabilizing the tetrahedral structure of the hydrate in the enzyme-inhibitor complex has been suggested based on an X-ray crystal structure of a hydrated trifluoromethyl ketone that showed intramolecular hydrogen bonding between the S atom and the –OH group on the carbonyl of the hydrate [70]. Such trifluoromethyl ketones are putative transition state esterase inhibitors, thought to act by forming a tetrahedral covalent hydrate with the catalytically active serine of carboxylesterases.

In conclusion, by using the inaccessible solid angle $_h$ calculated around different atoms as a novel measure of steric hindrance, a QSMR equation could be developed that can account for a large part of the variance in the log half-lives of a variety of noncongener carboxylic ester-containing drugs. In agreement with a recently proposed mechanism for hydrolysis by carboxylesterases, the corresponding equation suggests that steric hindrance around the sp^2 oxygen and charge on the sp^2 carbon of the ester moiety have the most important influence on the rate of *in vitro* human blood enzymatic hydrolysis.

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