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An ab Initio Study of Electronic Factors in Metabolic Hydroxylation of Aliphatic Carbon Atoms

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The monooxygenase-mediated hydroxylations of aliphatic carbon atoms are known to be regioselective for positions α to heteroatoms or to π systems (aromatic rings, carbon–carbon double bonds, carbonyl groups). Ab initio calculations (STO-3G and in some cases 4-31G) were performed on model molecules, indicating that the Mulliken overlap populations (taken as indices of electron bond densities) of C_α–H bonds being regioselectively hydroxylated are larger than C_β–H and C_γ–H overlap populations. These results support the hypothesis that metabolic C-hydroxylations occur by insertion of an activated oxygen species of electrophilic nature, probably oxene.

Carbon atoms in drugs and other xenobiotics undergo monooxygenase-mediated oxidations;[2] many questions regarding the basic mechanisms of these reactions cannot be answered convincingly at present, while it is certain that innumerable other questions remain unformulated.

In the past years, much effort has been directed toward an understanding of oxygen activation by monooxygenases.[3] These enzymes act by cleaving one molecule of oxygen and transferring an activated oxygen species to the substrate. There is now converging[4-7] but not definitive[8] evidence that cytochrome P-450 monooxygenases transfer a highly electrophilic oxenoid species to the substrate. A scheme summarizing our current understanding of cytochrome P-450 mediated oxygen activation, oxygen cleavage, and oxene transfer has been published.[9] Flavin-cofactored monooxygenases also act as oxenoid reagents.[10,11]

The mechanism of activated oxygen transfer from monooxygenases to aliphatic carbon atoms in drugs and other xenobiotics has been investigated by stereochemical studies[12-14] (for reviews, see also ref 2 and 15). The results have shown that the reaction occurs by a frontside displacement involving the insertion of an oxygen atom in the C–H bond. Isotope kinetic studies confirm that the cleavage of the C–H bond is often rate limiting in the overall hydroxylation reaction[16-19] (for a review, see also ref 20). A transition state involving approach of the activated oxygen atom toward the C–H bond and creation of a 2-electron 3-center bond has also been postulated in N-deethylation reactions.[21] Recently, an elegant technique utilizing intramolecular competition has shown unexpectedly high primary kinetic isotope effects in benzylic hydroxylation catalyzed by cytochrome P-450.[22] These results are fully compatible with, and provide strong evidence for, the oxene model.

A significant structural feature of biotransformation processes is their regioselectivity; this is particularly marked with aliphatic carbon hydroxylations. Indeed, it is consistently found that aliphatic carbons in position α to a heteroatom (N, O, S), an aromatic ring (benzylic position), a carbon–carbon double bond (allylic position), or a carbonyl group undergo preferred hydroxylation as compared to carbon atoms in β, γ, or other positions. The topic of regioselective biotransformation has been extensively reviewed.[23] Recent studies of interest include the first report of enzymic hydroxylation α to an acetylenic group[24] and a careful investigation of the metabolism of 1-methylcyclohexene and related terpinoids in mammals showing high regioselectivities for allylic positions.[25] The biotransformation of *N*-*n*-propylamphetamine by rat liver homogenates yielded ten times more N-dealkylated metabolites (resulting from C_α-hydroxylation) than C_β-hydroxylated metabolites.[26]

A recent quantum mechanical study[9] using the EHT method has pointed to a direct correlation between the electron density at the C–H bond and the regioselectivity of metabolic oxidation. Indeed, the C–H electron density at the benzylic position and at carbon atoms α to nitrogen and oxygen atoms was found to be larger than the C–H electron density at other positions. In the present study, these preliminary results are confirmed by ab initio calculations (STO-3G and 4-31G) and extended to include other types of C_α positions.

Computations. The molecules investigated belong to three groups: the *N*-alkyl derivatives [ethylamine (**1**), *n*-propylamine (**2**), protonated *n*-propylamine (**2**$^+$-H), isopropylamine (**3**), and *N*-ethylaniline (**4**)]; the *O*-alkyl derivatives [ethyl methyl ether (**5**), *n*-propyl methyl ether (**6**), isopropyl methyl ether (**7**), and phenethole (**8**)], and the π-alkyl group [ethylbenzene (**9**), 1-butene (**10**), 1-butyne (**11**), and propionamide (**12**)]. Their oxidative metabolism is known either for the molecules as such or as fragments of larger molecules.[2] Standard geometries were used,[27,28] and no geometry optimization was undertaken. The molecules were set in low-energy conformations as depicted (**1**–**12**). The neutral (nonprotonated) state was considered for the amines, with the exception of **2**$^+$-H taken for comparison purposes. Kinetic studies have indeed revealed that the basic amines undergoing N-demethylation react in the neutral state with microsomal enzymes.[29]

The ab initio SCF–MO calculations[30] were carried out on the CDC-CYBER 7326 computer of the Federal Institute of Technology and of the University of Lausanne using the GAUSSIAN 70 program.[31] In this treatment, each molecular orbital is represented as a linear combination of a set of basis functions. Current computations replace the Slater-type (exponential) functions with linear combinations of Gaussian functions. When the basis functions correspond just to those shells which are fully or partly populated in the atomic ground state, the basis set is described as minimal. The most popular basis set is

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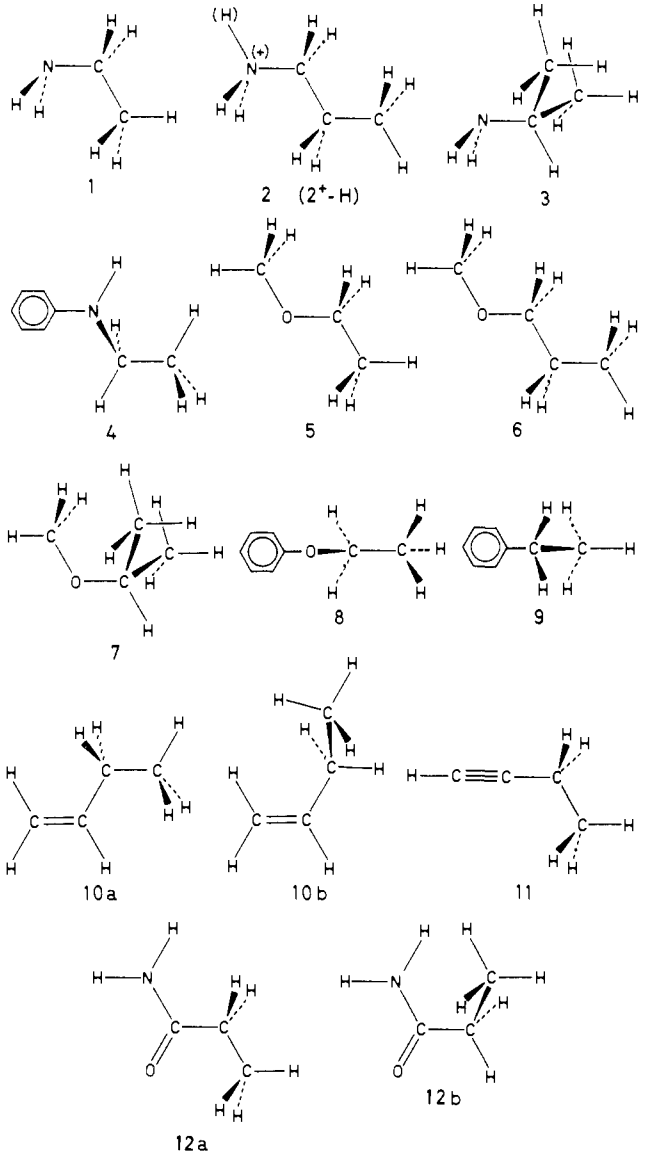

termed STO-3G and comprises three Gaussian functions per Slater-type orbital. To provide extra flexibility for the molecular orbitals, the use of additional functions is often required, a common procedure being to replace each minimal basis function by two functions, namely an inner and an outer part. In the split-valence-shell 4-31G basis, the inner shell (1s) of the first-row atoms is represented by a fixed sum of four Gaussian functions, while the valence shell (2s, 2p) is divided into "inner" and "outer" components represented by three and one Gaussians, respectively. This slight increase over a minimal basis set is a step toward the Hartree–Fock limit (infinitely flexible basis set), and agreement with experiment is most generally found to increase when going from a STO-3G level to a 4-31G level. The improvement is particularly clear when considering electronic distribution. Indeed, by allowing a greater and more realistic flexibility to the molecular orbitals, the 4-31G basis set is less restrictive than the minimal basis set in terms of electron density differences within a molecule; also, energy differences between various molecular structures are likely to be determined more accurately.[32,33]

In the present study, the STO-3G minimal basis set is used to calculate charge and bond densities in the model molecules. The calculations are then run at the 4-31G level for some significant molecules in order to verify the STO-3G results.

Results and Discussion

In the molecules investigated, the charge densities at carbon atoms selectively hydroxylated and at adjacent hydrogens were considered; they did not show any consistent trend which would differentiate these atoms from less biologically reactive centers. On the other hand, simple EHT calculations[9] have shown that the Mulliken overlap population of the C–H bonds consistently parallels the metabolic regioselectivity for the benzylic position and for carbon atoms α to a nitrogen or an oxygen atom. The ab initio results for the aliphatic and aromatic amines are presented in Table I. The minimum basis set STO-3G yields larger Mulliken overlap population (taken as indices of electron densities) in the C_α bonds as compared to the C_β–H and C_γ–H bonds. These differences are only moderate and may not appear as significant. In order to obtain a more realistic value for the electron density difference between C_α–H and C_β–H bonds, ethylamine was also studied using the 4-31G basis set. The results (Table I) show that STO-3G markedly underestimates this difference and that the C_α–H bond density is, in fact, considerably larger than that of the C_β–H bond.

In the case of *O*-alkyl groups, the STO-3G calculations show C_α–H electron bond densities to be almost identical or somewhat smaller than those at C_β–H and C_γ–H bonds. These results contradict our hypothesis, and their reliability has been tested by 4-31G calculations of ethyl methyl ether (**5**). Table II shows that the more extended basis set distinctively indicates the C_α–H bond densities to be larger than the C_β–H densities. The difference, however, is not as large as with *N*-alkyl groups. In this context, it is interesting to note that globally *O*-alkyl groups may well experience a less marked regioselectivity than *N*-alkyl groups. Compare, for example, the case of *N*-*n*-propylamphetamine discussed above[26] and the metabolism of *n*-propyl *p*-nitrophenyl ether using rat liver preparations; the latter studies indeed showed that C_α- predominated over C_β-hydroxylation after induction only.[34]

The results for the molecules containing an alkyl group ($-CH_2CH_3$) adjacent to an unsaturated system are presented in Table III. Among these molecules, propionamide (**12**) has been chosen as a model for lactams, such as cotinine,[35] which undergo selective α-hydroxylation. The STO-3G calculations indicate larger C_α–H than C_β–H electron bond densities for ethylbenzene (**9**) and 1-butyne (**11**). The reverse holds for 1-butene (**10**) and propionamide (**12**) (extended and folded conformations). To examine the validity of these results, 4-31G calculations have been performed for 1-butene (**10**) and 1-butyne (**11**). As with amines and ethers, the latter calculations show the STO-3G basis set to be in error when predicting smaller C_α–H than C_β–H bond densities and also to underestimate the C_α–H minus C_β–H density difference, when this difference is positive. The 4-31G results for 1-butyne are particularly spectacular, showing a large bond density difference and a marked variation as compared to the STO-3G calculations.

Experimental regioselectivity studies based on intramolecular competition for benzylic positions have shown that electron-withdrawing para substituents decrease benzylic hydroxylation.[36] This finding confirms the electrophilic nature of benzylic hydroxylation also indicated by the present calculations of ethylbenzene (**9**) (Table III).

Conclusion

In the present study, the experimentally characterized regioselectivities are shown to be correlated with comparably high C–H Mulliken overlap populations. This

Table I. Mulliken Overlap Populations of Aliphatic C-H Bonds in *N*-Alkyl Derivatives

Molecule	STO-3G			4-31G	
	C_α-H	C_β-H	C_γ-H	C_α-H	C_β-H
Ethylamine (1)	0.774	0.765[a]		0.802	0.736[a]
	0.774	0.767		0.802	0.765
		0.767			0.765
Propylamine (2)	0.774	0.765	0.769		
	0.774	0.765	0.769		
			0.769		
Propylammonium cation (2^+-H)	0.782	0.768	0.773[a]		
	0.782	0.768	0.770		
			0.770		
Isopropylamine (3)	0.769	0.769[b]			
		0.767			
		0.767[c]			
N-Ethylaniline (4)	0.778[d]	0.769[e]			
	0.770	0.768			
		0.768			

[a] Hydrogen in the plane of the heavy atoms. [b] Hydrogen anti to the methenyl H. [c] Hydrogen anti to the heteroatom. [d] Hydrogen anti to N-H. [e] Hydrogen closest to plane of ring.

Table II. Mulliken Overlap Populations of Aliphatic C-H Bonds in *O*-Alkyl Derivatives

Molecule	STO-3G				4-31G		
	C_α-H				C_α-H		
	($-CH_3$)	$-CH_2-$	C_β-H	C_γ-H	($-CH_3$)	$-CH_2-$	C_β-H
Ethyl methyl ether (5)	(0.769[a])	0.758	0.768		(0.790[a])	0.778	0.754[a]
	(0.756)	0.758	0.768		(0.760)	0.778	0.771
	(0.756)		0.768		(0.760)		0.771
Propyl methyl ether (6)	(0.769[a])	0.757	0.766	0.770[a]			
	(0.756)	0.757	0.766	0.768			
	(0.756)			0.768			
Isopropyl methyl ether (7)	(0.769[a])	0.773	0.772[b]				
	(0.760)		0.768				
	(0.760)		0.764[c]				
Phenethole (8)		0.760	0.768				
		0.760	0.768				
			0.768				

[a-c] See corresponding footnotes in Table I.

Table III. Mulliken Overlap Populations of Aliphatic C-H Bonds in π-Alkyl Derivatives

Molecule	STO-3G		4-31G	
	C_α-H	C_β-H	C_α-H	C_β-H
Ethylbenzene (9)	0.775	0.767[b]		
	0.775	0.769		
		0.769		
1-Butene extended (10a)	0.763	0.769	0.795	0.778[a]
	0.763	0.769	0.795	0.777
		0.769		0.777
1-Butene folded (10b)	0.769[c]	0.770[d]	0.794[c]	0.779[d]
	0.763	0.769	0.796	0.777
		0.768[e]		0.765[e]
1-Butyne (11)	0.773	0.770	0.827	0.771[a]
	0.773	0.770	0.827	0.778
		0.770		0.778
Propionamide extended (12a)	0.760	0.769[a]		
	0.760	0.770		
		0.770		
Propionamide folded (12b)	0.770[c]	0.768[f]		
	0.759	0.770		
		0.770[e]		

[a] See corresponding footnote in Table I. [b] Hydrogen anti to phenyl ring. [c] Hydrogen in plane of π system. [d] Hydrogen closest to C=C. [e] Hydrogen anti to (closest) sp^2 carbon. [f] Hydrogen closest to N.

provides a strong theoretical support to the electrophilic nature of monooxygenase-mediated aliphatic carbon hydroxylation and to the mechanism of oxene insertion into C-H bonds postulated by several authors. It must be noted, however, that the correlation established between regioselectivity and C-H bond density is only of a qualitative nature. Indeed, the C-H electron bond densities depend heavily on the MO model used; also, the degree of metabolic regioselectivity varies with the biological system used (species, organ, induction, etc.). It would therefore be prematurate to expect quantitative predictions from the present or similar studies. Rather, the above results put qualitative (intuitive) predictions on a firmer basis and allow mechanistic insights.

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Notes

Electronic Structures of Some Antimicrobial *N*-Chloramines. Possible Existence of Intramolecular Hydrogen Bonding and Its Effect on Germicidal Efficiency

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The photoelectron spectra of eight *N*-chloramines and *N,N*-dichloramines derived from either α-aminoisobutyric acid or 2-amino-2-methylpropanol have been measured. The lone-pair ionization potentials obtained from the photoelectron spectra have been interpreted to indicate that a substantial intramolecular interaction exists between the N–H function and the various oxygen lone pairs of the *N*-chloramines. Such an intramolecular interaction for the *N*-chloramines can explain at least in part why these molecules are less potent as antimicrobial agents than are their *N,N*-dichloramine analogues for which a similar intramolecular interaction is impossible.

Comparative antimicrobial studies of *N*-chloramines and *N,N*-dichloramines derived from α-aminoisobutyric acid or 2-amino-2-methylpropanol have shown that the antimicrobial activities of the compounds are influenced by several factors, including the degree of chlorination, the presence of denaturant, and the polarity of the N–Cl bond.[1] Of particular interest are the observations[1] that *N*-chloramines with highly polar N–Cl bonds are readily denatured, whereas those with relatively nonpolar N–Cl bonds are not, and that *N,N*-dichloramines have antimicrobial activities which are virtually unchanged by the presence of denaturant. This lack of deactivation by denaturant is supportive of the observation that *N,N*-dichloramines are more effective antimicrobial agents than are their *N*-chloramine analogues.

Ultraviolet photoelectron spectroscopy is the best means available for studying the electronic structures of complex molecules. It is particularly useful for investigating the energies and interactions of nonbonding ("lone pair") electrons, for the nonbonding molecular orbitals usually lie at somewhat higher energies than do the bonding MO's and thus normally give rise to identifiable, well-resolved, low-energy ionization bands in the photoelectron spectra. A detailed study of the lone-pair bands in the spectra of the *N*-chloramines and *N,N*-dichloramines should be especially illuminating given that the polarity of the N–Cl function is deemed to be of importance in determining the activity of the antimicrobial agents. It has been demonstrated previously that photoelectron spectroscopy is a viable tool for rationalizing pharmacological activity.[2,3]

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