Molecular Modelling Study of the Lidocaine, Procaine, and Their Metabolites

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Received 30 January 1995

The AM1 quantum-chemical method has been used to determine stable structures and proton affinities of local anaesthetics lidocaine, procaine, and their main metabolites. The reaction enthalpies for the deethylation and hydroxylation of the lidocaine and the hydrolysis of procaine were computed. The lipophilic properties of these compounds were also investigated. The observed relative activities of lidocaine and its active metabolites correlated with both the calculated proton affinity and lipophilicity of these compounds.

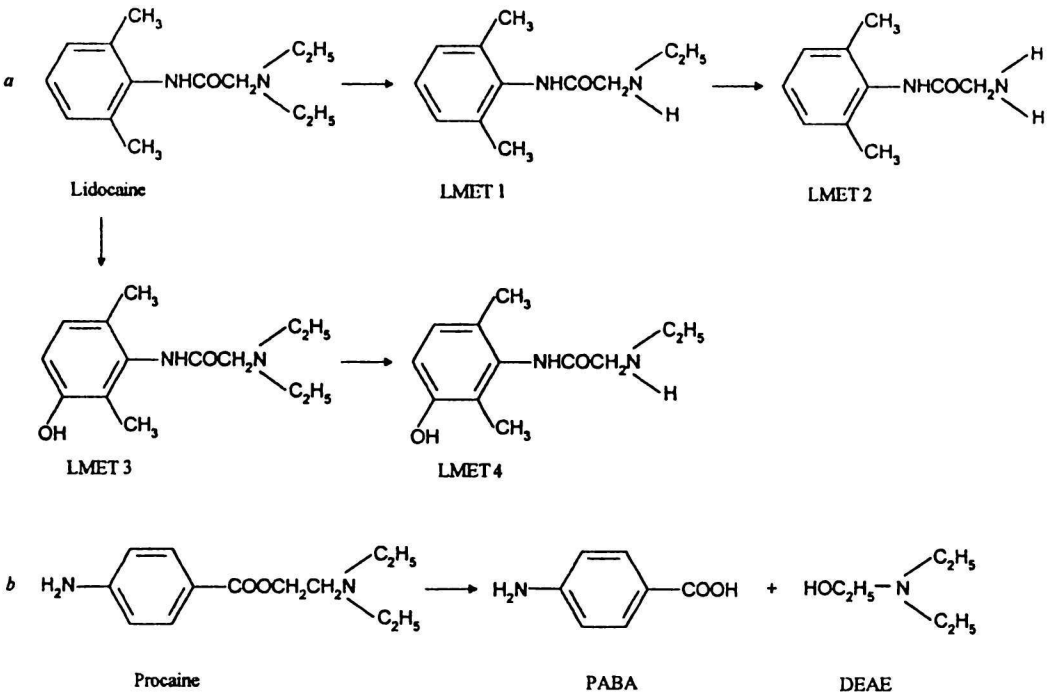
Procaine and lidocaine are well known clinically used local anaesthetics (LA) [1]. Lidocaine also exhibits considerable antiarrhythmic activity [2]. These compounds, like many other neuroactive drugs, must penetrate into or across the neuronal plasmalemma to be pharmacologically active [2]. In order to explain the molecular mode of actions of these drugs, the effect of procaine and lidocaine on the cell membranes was intensively experimentally investigated [3—6]. Based on the premise that the knowledge of a detailed three-dimensional structure of these drugs must be important for the explanation of the structure—activity relationships, some authors devoted their work to the investigation of the molecular structure of procaine [7—10] and lidocaine [11—15].

The use of these drugs may be complicated by the presence of active metabolites [16, 17]. The metabolic fate of the procaine and lidocaine has been extensively studied [16—19] and principal metabolites were determined [16, 18—21] (Scheme 1). It is assumed that procaine metabolites interact with neuronal voltage-gated Na^+ channels in a manner similar to that of procaine and other local anaesthetics [16]. Similarly, it is shown [17] that also metabolites of the lidocaine-type antiarrhythmics can bind to this class drug receptor associated with the sodium channel. Considering the chemical structures of LA's there are several loci on the channel where LA is likely to bind. However, none of these have been definitely identified using the techniques of molecular biology. The absence of three-dimensional structural data for transmembrane receptors presents a challenge to the application of molecular modelling methods to gain insights into the recognition and binding process. The results of theoretical modelling [22—29] of interactions of associative sites of LA's with polar groups (as carboxylate, phosphate, amine, amide) of membranes have been used to identify molecular determinants of recognition and binding process. The effect of medium on the equilibrium geometry and interaction energy of the LA—carboxylate complexes was investigated [29]. Effect of hydration on stable conformations of LA has also been studied [30].

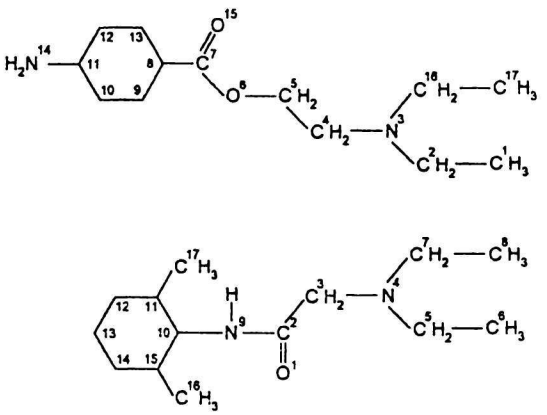
Based on these results we applied the methods of theoretical chemistry to a group of two most frequently used LA's (procaine, lidocaine) and their six metabolites. Of particular interest were physicochemical properties of these species (structural characteristics, proton affinity, reaction enthalpy, partition coefficient) and how these parameters correlate with available experimental (physicochemical and biological) data. The calculations reported here also represent the first molecular modelling study of procaine and lidocaine metabolites.

THEORETICAL METHODS

The geometries of procaine and its metabolites *para*-aminobenzoic acid (PABA) and diethylaminoethanol (DEAE), lidocaine and its metabolites ω-diethylamino-2,6-dimethylacetanilide (LMET1), ω-amino-2,6-dimethylacetanilide (LMET2), 3-hydroxy-ω-diethylamino-2,6-dimethylacetanilide (LMET3), and 3-hydroxy-ω-ethylamino-2,6-dimethylacetanilide (LMET4) (Scheme 1) were fully energy-optimized using the quantum-chemical MO-SCF AM1 method [31]. The molecular graphic and molecular modelling studies of the AM1 optimized structures were carried out by means of the MOLGEN 3.0 program [32]. For the calculation of partition coefficients the MGP program [33] was used. The labeling of the atoms of procaine and lidocaine is shown in Scheme 2.



Scheme 1. Metabolic pathways for lidocaine (*a*) and procaine (*b*).



Scheme 2. Numbering of atoms in procaine and lidocaine.

The semiempirical quantum-chemical AM1 method allows the calculation of the standard ($T = 298$ K) formation enthalpies [34], $\Delta H^0_{f,298}$. The proton affinity of base PA(B) can be computed by the equation

$$\mathrm{PA(B)} = \Delta H^0_{f,T}(\mathrm{H^+, g}) + \Delta H^0_{f,T}(\mathrm{B, g}) + \Delta H^0_{f,T}(\mathrm{BH^+, g}) \quad (1)$$

$\Delta H^0_{f,T}$ represents the heat of formation of the species stated between parenthesis. For $\Delta H^0_{f,298}(\mathrm{H^+, g})$ the experimental value 1537.1 kJ mol^{-1} is taken [35]. All quantum-chemical calculations were performed using the AMPAC program [36].

RESULTS AND DISCUSSION

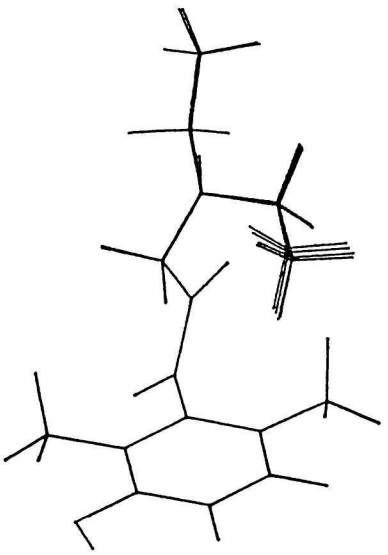
Geometry

The quantum-chemical calculations of conformational energy maps [7, 8, 13, 30] reveal the presence of several stable conformations in basic and protonized LA's. Without taking into account any environment, the most stable conformations correspond to bent structures stabilized by intramolecular hydrogen bonds. Among the several binding possibilities of these drugs to the receptor the strongest interaction is that of ion-pair type [22—29]. The actual, biologically active conformation may be better represented by structures observed in the ion pairs of some LA's. Therefore, the thermodynamically stable X-ray structures of lidocaine [37] and procaine [38] hydrochlorides were considered as starting geometries for the AM1 energy optimizations of drugs in this study.

The structures of lidocaine and its four metabolites were fully optimized by the AM1 method. Selected AM1 geometric values for the lidocaine and its metabolites are listed in Table 1. As it is seen from this table the deethylation and/or hydroxylation practically does not change the overall mutual arrangement of the hydrophobic (aromatic) part and hydrophilic (amine) group of the drug. The torsion angle Ψ defining the orientation of the aromatic and amide groups, is practically the same in all compounds studied and its value is close to those found in different crystal environments (67.9—76.9°, Refs. [37, 39, 40]). The amide group is in all compounds investigated nonplanar, but the deviation from planarity is small (8—10°,

Table 1. Selected AM1 Optimized Parameters for Lidocaine and its Metabolites

	Lidocaine		LMET1	LMET2	LMET3	LMET4
	AM1	X-Ray [39]				
			Bond lengths r/pm			
O-1—C-2	124.5	122.2	124.5	124.5	124.4	124.5
C-2—C-3	154.3	151.4	154.2	154.3	154.2	154.3
C-3—N-4	144.3	146.7	143.8	143.5	144.2	143.8
C-2—N-9	138.9	134.3	138.7	138.6	139.1	138.9
N-9—C-10	141.5	143.2	141.4	141.5	141.4	141.4
			Bond angles Φ/°			
O-1—C-2—N-9	122.5	125.1	122.9	123.3	122.5	123.0
C-3—C-2—N-9	115.7	113.4	116.1	116.4	115.6	116.0
C-2—C-3—N-4	116.9	114.5	115.7	115.3	117.0	115.7
C-2—N-9—C-10	124.6	122.3	124.9	124.8	124.7	124.8
			Torsion angles Ψ/°			
C-15—C-10—N-9—C-2	66.0	67.9	65.5	66.1	65.8	65.7
C-10—N-9—C-2—O-1	351.7	353.8	351.1	350.5	351.3	351.0
N-9—C-2—C-3—N-4	157.2	149.7	158.5	159.1	158.5	159.2
C-2—C-3—N-4—C-5(H-5)	67.3	79.0	57.8	60.1	67.7	54.8



Scheme 3. The superposition of the AM1 optimized conformers of lidocaine and its four metabolites studied.

Table 2. Selected AM1 Optimized Parameters for Procaine

	Procaine	
	AM1	X-Ray [38]
	Bond lengths r/pm	
C-8—C-7	146.4	146.8
C-7—O-15	123.6	121.6
C-7—O-6	137.3	136.1
O-6—C-5	143.5	143.9
C-5—C-4	153.3	150.2
C-4—N-3	144.7	150.8
	Bond angles Φ/°	
C-8—C-7—O-6	114.2	112.2
C-8—C-7—O-15	128.2	127.6
C-7—O-6—C-5	116.4	116.4
O-6—C-5—C-4	106.9	109.2
C-5—C-4—N-3	118.4	116.3
C-4—N-3—C-2	112.9	112.0
	Torsion angles Ψ/°	
C-9—C-8—C-7—O-15	176.2	185.9
C-8—C-7—O-6—C-5	178.4	179.4
C-7—O-6—C-5—C-4	176.3	172.7
O-6—C-5—C-4—N-3	71.0	70.3
C-5—C-4—N-3—C-2	61.3	60.0

Table 1). A little bit lower deviation from planarity of the amide group (about 5—6°) was also observed in the crystals of lidocaine salts [37, 39, 40]. The N-9—C-2—C-3—N-4 fragment adopts the nonplanar *trans* conformation in both lidocaine and its metabolites with very close torsion angles (Table 1). The very low deviations of the structures of metabolites from the optimized lidocaine conformer are also manifested in Scheme 3. This scheme was prepared by means of the molecular graphics program MOLGEN 3.0 and represents the superposition of the lidocaine metabolites studied with respect to the lidocaine. The superposition was carried out with respect to the atoms C-10, C-11, and N-9, respectively. A goodness of fit was expressed in terms of Root Mean Square (RMS) value (equal to 0.001). The major features of the geometry predicted by AM1 for the lidocaine are in agreement with those of the previous X-ray study [39] (Table 1).

The selected bond lengths, bond angles, and torsion angles, together with the experimental X-ray values [38] of the procaine hydrochloride are given in Table 2. In general, the values obtained with the AM1 method and experimental data are very similar. However, several differences are seen. The length C-4—N-1 was computed considerably shorter and the length

Table 3. AM1 Calculated A, B, and C Interatomic Distances (in pm), Proton Affinities, and Relative Activity (RA) of the Compounds Studied

Compound	A($C_{arom}\cdots O$)		B($N\cdots O$)		C($C_{arom}\cdots N$)		PA/(kJ mol^{-1})	RA[b]
	B	BH^+	B	BH^+	B	BH^+		
Lidocaine	287	288	290	282 297[a]	504	499 485[a]	916.7	100
LMET1	288	290	285	270	499	497	900.8	85
LMET2	288	291	282	267	498	495	879.0	26
LMET3	288	290	285	270	499	497	915.5	
LMET4	287	289	290	288	501	500	900.0	
Procaine	243	244	493	492 497[a]	523	525 521[a]	935.2	
DEAE							933.1	

a) X-Ray data; *b*) Relative activity in man with respect to the lidocaine (Refs. [44, 45]).

C-5—C-4 longer than the experimental values. As regards bond angles, the largest difference between theory and experiment (2.3°) was observed for the O-6—C-5—C-4 angle. As follows from the comparison of the torsion angles (Table 2) the stereochemistry of the C—CO—C—C—N connecting chain of procaine is in the optimized structure close to those found in the crystal of procaine hydrochloride [38]. The carboxylic group of procaine is calculated to be practically coplanar with the aromatic ring. The C-8—C-7—O-6—C-5 and C-7—O-6—C-5—C-4 fragments exist predominantly in the planar arrangement. On the other hand, the O-6—C-5—C-4—N-3 and C-5—C-4—N-3—C-2 groups are in stable *gauche* conformations.

For more quantitative description of the structural differences of both drugs we also determined the intermolecular distances A($C\cdots O$), B($N\cdots O$), and C($C\cdots N$) connecting the nonbonding nitrogen, oxygen, and aromatic carbon (C-8, C-10) atoms, respectively, specifying the separation of the two polar groups of drugs able to interact with the corresponding binding sites of the membrane [24—28] (Table 3). The analysis of these separations among the common functional groups of LA's and their metabolites reveals that they are within certain narrow intervals. However, the interatomic separations between amine and oxygen atoms are very different for lidocaine (282 pm) and procaine (492 pm). This suggests that the binding sites for these two drugs should be different. The calculated analogous distances for metabolites of the lidocaine are very close to those found for parent drug (Table 3). Thus the common pharmacophoric patterns of lidocaine and its metabolites indicate that these species may bind to the same receptor. *Sheldon et al.* [17] have recently shown experimentally that antiarrhythmics of the lidocaine type and their metabolites bind to the same antiarrhythmic receptor. This is not surprising given that our fully optimized structures of lidocaine metabolites are very similar to that of their parent drug (Table 3, Scheme 3).

Proton Affinity and Reaction Enthalpy

At physiological pH, procaine, lidocaine, and their metabolites can occur in positively charged or uncharged forms. The protonation site is the amino group. Table 3 summarizes the AM1 calculated proton affinities (PA) of compounds studied. For evaluation of proton affinities the fully geometry-optimized structures of base and corresponding cation were used. The highest values of PA were computed for the tertial amines and with the decreasing alkylation the PA also decreases (Table 3). The same dependence was observed experimentally for vapour phase protonation of methyl-substituted ammonia [41]. The experimental gas-phase PA's for methylamine, dimethylamine, and trimethylamine (896.2 kJ mol^{-1}, 923.0 kJ mol^{-1}, and 944.4 kJ mol^{-1}) (Ref. [41]) are in general agreement with corresponding AM1 calculated PA's (Table 3) for lidocaine and procaine derivatives investigated. The computed proton affinities correlate with the relative activities of lidocaine and its active metabolites (Table 3). A good correlation ($R = 0.971$) was found between the relative activity and proton affinity of these three agents. The very close PA computed for the procaine and its simpler metabolite diethylaminoethanol (Table 3) shows that the side aromatic parts of the molecule do not significantly influence the basicity of the hydrophilic amino group of drug. The practically equal basicity of procaine and its metabolite diethylaminoethanol (DEAE) is probably responsible for the fact that DEAE, after intravenous injection, shows many actions of procaine [16]. Thus it is possible that DEAE interacts with neuronal Na^+ channels in a manner similar to that of procaine.

The reaction enthalpies for the deethylation and hydroxylation of the lidocaine and the hydrolysis of the procaine are shown in Table 4. Although these calculations do not take into account the entropy changes and effect of medium, it was hoped that in comparison with the same reaction for the hydrolysis and/or hydroxylation of the compounds studied these factors

Table 4. Reaction Enthalpies for the Reactions of the Lidocaine and Procaine

No.	Reaction	$\Delta H^0_{f,298}$ (kJ mol^{-1})
1	Lidocaine + H_2O ⟶ LMET1 + C_2H_5OH	−15.9
2	LMET1 + H_2O ⟶ LMET2 + C_2H_5OH	−1.3
3	Lidocaine + H_2O ⟶ LMET3 + H_2	46.9
4	LMET3 + H_2O ⟶ LMET4 + C_2H_5OH	−16.3
5	LMET1 + H_2O ⟶ LMET4 + H_2	46.5
6	Procaine + H_2O ⟶ PABA + DEAE	−18.4

Table 5. Calculated and Experimental Partition Coefficients, log *P*

Compound	log *P*	
	Calculated	Experimental [2]
Lidocaine	2.41	2.56
LMET1	1.69	
LMET2	0.84	
LMET3	2.02	
LMET4	1.30	
Procaine	1.73	2.0
PABA	0.79	
DEAE	0.40	

would at least partially cancel. For the deethylation (reactions *1*, *2*, and *4*) of the lidocaine and its metabolites the exothermic reactions with small absolute values of enthalpies are characteristic. The deethylation of the tertiary amine is energetically more favourable in comparison with the same reaction of the secondary amine (Table 4). The larger, but positive reaction enthalpies were found for the hydroxylation reactions *3* and *5* (Table 4). The computed enthalpies for deethylation and hydroxylation of lidocaine qualitatively correlate with the *in vivo* experiments. These experiments [21] have shown that the capacity of lidocaine 3-hydroxylation is small and saturable at low substrate concentrations while *N*-deethylation is not saturable. The hydrolysis of the procaine is exothermic with the calculated enthalpy of −18.4 kJ mol^{-1}. Another question arises as to whether there should be a barrier to the formation of reaction products. The activation energies for the reactions *1*—*6* (Table 4) are not known. Their magnitude may have (together with the catalytic influence of substrate) some influence on gas phase stability.

Hydrophobic Properties

Hydrophobicity of local anaesthetic is one of decisive physicochemical parameters which is responsible for its ability to penetrate a hydrophobic domain of the membrane. A clear correlation between potency and hydrophobicity is observed [42]. Because the base of local anaesthetic is more membrane-permeant, it accounts for most of the bulk passage of drug through membrane hydrophobic barriers [2]. Hydrophobicity of a drug is usually measured as *P*, the partition coefficient of the molecule in the water—octanol system. Table 5 contains the calculated log *P* of compounds under study using the MGP program. Log *P* was computed from the hydrophobic atomic parameters defined by *Crippen et al.* [43] and is in a very good agreement with the available experimental partition coefficients for lidocaine and procaine. The metabolites were calculated more hydrophilic than their parent drugs. Similarly, the nonactive hydroxylated metabolites of the lidocaine (LMET3 and LMET4) are more hydrophilic than the corresponding active compounds (lidocaine and LMET1). In man, LMET1 and LMET2 have 85 % (Ref. [44]) and 26 % (Ref. [45]) of the activity of lidocaine. Using regression analysis with calculated log *P* as the independent variable a very good correlation with the relative activity (RA) of lidocaine (100 %), LMET1 (85 %), and LMET2 (26 %) was obtained

$$\mathrm{RA} = -8.374 + 47.798 \pm 13.855 \log P \qquad (2)$$
$$R = 0.9602; \quad \mathrm{SE} = 15.443; \quad F = 11.832$$

As it is well known [2] the hydrophobicity of local anaesthetic significantly contributes to the potency of the drug, because it increases its accessibility to some functional sites in the membrane. The presence of the polar hydroxyl group on the aromatic part of the metabolites LMET3 and LMET4 considerably decreases the lipophilicity of this moiety of the compound which should prevent their penetration through the hydrophobic part of the membrane to the right place and create the effective interaction with the receptor in order to produce measurable pharmacological response.

CONCLUSION

This theoretical study was set out to determine physicochemical information about lidocaine and procaine metabolites and their parent compounds for which a relatively small amount of experimental structural data exists, considering their pharmacological importance. Using the molecular modelling methods the following conclusions can be drawn.

1. The structural diversity of lidocaine and procaine resulting from the molecular modelling studies indicates that these drugs could bind to different receptors. The deethylation and/or ring-hydroxylation of the lidocaine does not change the conformation of the side chain containing the —N(H)—C(O)—C—N< atoms and reveals the common pharmacophoric patterns of lidocaine and its metabolites.

2. The deethylation of lidocaine and the hydrolysis of procaine are in vapour state exothermic reactions. On the contrary, the hydroxylation of lidocaine is found to be highly endothermic.

3. A different computed partition coefficient, log P found for active and inactive metabolites of lidocaine should explain their different biological behaviour.

The data obtained in this work will be useful for further investigations of the mechanism of action of local anaesthetics on the molecular level.

Acknowledgements. Support of this work from the Slovak Ministry of Education (Grant No. 1/1224/94) and from ECC Program COPERNICUS (Grant No. ERB3512PL941009) is gratefully acknowledged.

REFERENCES

1. Wolff, M. E. (Editor), *Burger's Medicinal Chemistry*, 4th Edition, Parts *II* and *III*, *The Basis of Medicinal Chemistry*. Wiley, New York, 1979, 1981.
2. Strichartz, G. R., Sanchez, V., Arthur, G. R., Chafetz, R., and Martin, D., *Anesth. Analg. 71*, 158 (1990).
3. Courtney, K. R., *J. Pharmacol. Exp. Ther. 213*, 114 (1980).
4. Fink, B. R. (Editor), *Progress in Anesthesiology*, Vol. *1, 2*. Raven Press, New York, 1979, 1980.
5. Vaughan, Williams, E. M. (Editor), *Antiarrhythmic Drugs, Handbook of Experimental Pharmacology*, Vol. *89*. Springer-Verlag, Berlin, 1989.
6. Butterworth, J. F. and Strichartz, G. R., *Anesthesiology 72*, 711 (1990).
7. Coubeils, I. L. and Pullman, B., *Mol. Pharmacol. 8*, 278 (1972).
8. Gerhards, J., Ha, T. K., and Perlia, X., *Arzneim.-Forsch./Drug Res. 36*, 861 (1986).
9. Kashino, S., Ikeda, M., and Haisa, M., *Acta Crystallogr., B 38*, 1868 (1982).
10. Alcolea Palafox, M., *Theochem, J. Mol. Struct. 262*, 7 (1992).
11. Hanson, A. W. and Banner, D. W., *Acta Crystallogr., B 30*, 2486 (1974).
12. La Planche, L. A., Vanderkooi, G., Jasmani, H., and Suki, M. M., *Magn. Reson. Chem. 23*, 945 (1985).
13. Remko, M., *Collect. Czech. Chem. Commun. 51*, 2063 (1986).
14. Pattahiraman, N., Langridge, R., and La Planche, L. A., *J. Chem. Soc., Perkin Trans. 2 1989*, 1.
15. McMaster, P. D., Noris, V. J., Stankard, Ch. E., Byrnes, E. W., and Guzzo, P. R., *Pharm. Res. 8*, 1013 (1991).
16. Butterworth, J. F., Lief, P. A., and Strichartz, G. R., *Anesthesiology 68*, 501 (1988).
17. Sheldon, R., Thakore, E., Wilson, L., and Duff, H., *J. Pharmacol. Exp. Ther. 269*, 477 (1994).
18. Beckett, A. H., Boyes, R. N., and Appleton, P. J., *J. Pharm. Pharmacol. 18* Suppl., 76s (1966).
19. Thomas, J. and Meffin, P., *J. Med. Chem. 15*, 1046 (1972).
20. Nelson, S. J., Garland, W. A., Breck, G. D., and Trager, W. F., *J. Pharm. Sci. 66*, 1180 (1977).
21. Kawai, R., Fujita, S., and Suzuki, T., *J. Pharm. Sci. 74*, 1219 (1985).
22. Remko, M. and Van Duijnen, P. T., *Theochem, J. Mol. Struct. 104*, 451 (1983).
23. Remko, M. and Scheiner, S., *J. Pharm. Sci. 77*, 304 (1988).
24. Remko, M., *Theochem, J. Mol. Struct. 201*, 287 (1989).
25. Remko, M. and Scheiner, S., *J. Pharm. Sci. 80*, 328 (1991).
26. Remko, M., Scheiner, S., and Van Duijnen, P. T., in *QSAR in Design of Bioactive Compounds*. (Kuchar, M., Editor.) Pp. 83—95. Prouss, J. R. Science Publ., S. A., Barcelona, 1992.
27. Remko, M. and Rode, B. M., *J. Chem. Soc., Faraday Trans. 1 89*, 1321 (1993).
28. Remko, M., Scheiner, S., and Rode, B. M., *Theochem, J. Mol. Struct. 307*, 35 (1994).
29. Remko, M., Liedl, K. R., and Rode, B. M., *Theochem, J. Mol. Struct. 343*, 141 (1995).
30. Remko, M. and Sekerka, I., *Collect. Czech. Chem. Commun. 52*, 1879 (1987).
31. Dewar, M. J. S., Zoebisch, E. G., Healy, E. F., and Stewart, J. J. P., *J. Am. Chem. Soc. 107*, 3902 (1985).
32. Baričič, P. and Mackov, M., MOLGEN distributed by Baričič Software, 900 01 Modra, Slovakia, 1994.
33. Ertl, P., *Chem. Listy 86*, 465 (1992).
34. Voets, R., Francois, J. P., Martin, J. M. L., Mullens, J., Yperman, J., and Van Poucke, K. C., *J. Comput. Chem. 10*, 449 (1989).
35. Stull, D. R. and Prophet, H. (Editors), *JANAF Thermochemical Tables*. Natl. Stand. Ref. Data Ser., Natl. Bur. Stand., NSRDS-NBS 37, U.S. Govt. Print. Off., Washington, D.C., 1971.
36. Available from *Quantum Chemistry Program Exchange (QCPE)*, Program No. 506. Indiana University, Bloomington, Indiana.
37. Hanson, A. W. and Röhr, M., *Acta Crystallogr., B 28*, 3567 (1972).
38. Dexer, D. D., *Acta Crystallogr., B 28*, 77 (1972).
39. Yoo, C. S., Abola, E., Wood, M. K., Sax, M., and Pletcher, J., *Acta Crystallogr., B 31*, 1354 (1975).
40. Hanson, A. W., *Acta Crystallogr., B 28*, 672 (1972).
41. Aue, D. H., Webb, H. B., and Bowers, M. T., *J. Am. Chem. Soc. 98*, 311 (1976).
42. Gupta, S. P., *Chem. Rev. 91*, 1109 (1991).
43. Ghose, A. K., Pritchett, A., and Crippen, G. M., *J. Comput. Chem. 9*, 80 (1988).
44. Thomson, P. D., Elliott, H. L., Kelman, A. W., Meredith, P. A., and Whiting, B., *J. Pharmacokinet. Biopharm. 15*, 101 (1987).
45. Strong, J. M., Mayfield, D. E., Atkinson, A. J., Jr., Burris, B. C., Raymon, F., and Webster, L. T., *Clin. Pharmacol. Ther. 17*, 184 (1975).

Translated by M. Remko