Conformational Analysis of Novel Monoaryl Substituted Protoporphyrins by 1D NOE, 2D NOESY and Molecular Modeling Studies

Adriana E. Robinsohn and Graciela Y. Buldain

Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, (1113) Buenos Aires, Argentina

Marta S. Maier*

Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, (1428) Buenos Aires, Argentina

Received June 5, 1996
Revised June 2, 1997

Porphyrins **1-4** are monoaryl protoporphyrins substituted on C-5 or C-15 by a phenyl group. One- and two-dimensional nOe experiments and molecular modeling studies allowed us to find the most favorable conformations for these compounds. In the four porphyrins, the exocyclic phenyl group adopts a non-coplanar disposition relative to the plane of the macrocycle and this is reflected in the 1D nOe difference and 2D NOESY results. In porphyrins **1** and **3** the macrocycle is nearly planar while nonplanar saddle conformations were obtained for porphyrins **2** and **4**.

J. Heterocyclic Chem., **34**, 1339 (1997).

Introduction.

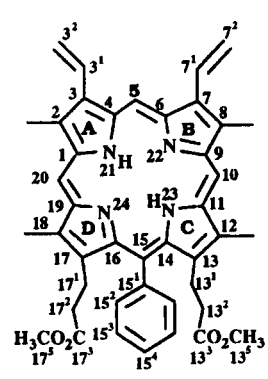
Horseradish peroxidase is a hemo (Fe-protoporphyrin IX)-protein which catalyzes redox reactions, employing as oxidizing agent the hydrogen peroxide generated *in vivo* as a by-product of enzymatic processes [1,2].

In order to study the topology of the active horseradish peroxidase site, X-ray crystallographic techniques were attempted, but suitable crystals could not be obtained [3]. However, valuable information was gained by treatment of horseradish peroxidase with alkylating agents such as arylhydrazine, obtaining partial attachment of the phenyl group to the 20-*meso* carbon together with partial conversion of the heme to its 18-hydroxymethyl derivative with subsequent loss of catalytic activity [4].

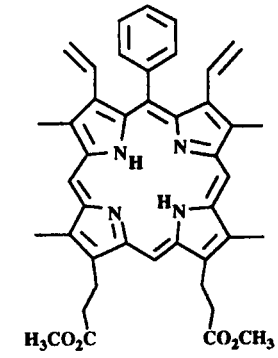
In an attempt to confirm whether alkylation only at the 20-*meso* carbon of the horseradish peroxidase prosthetic group induces enzymatic inactivation, syntheses were carried out [5] and conformational studies performed of four porphyrins isomeric with natural protoporphyrin IX substituted with a phenyl group at C-5 or C-15 (Scheme 1). Such synthetic hemines must then be recombined with horseradish peroxidase apoprotein in order to determine their catalytic activity.

X-ray crystallography and computational studies [6-8] of highly substituted porphyrins have shown that the porphyrin macrocycle is conformationally flexible and capable of adopting nonplanar conformations in order to minimize unfavorable steric interactions on the periphery of the macrocycle. These conformational variations are reflected in the spectroscopic properties of porphyrin derivatives [9] and may play an important role in controlling the biochemical functions of tetrapyrroles in proteins [10,11].

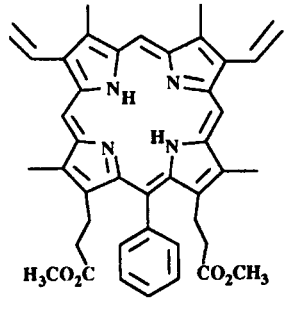
Scheme 1
Molecular Structures and Number for *meso*-Phenyl Protoporphyrins **1-4**



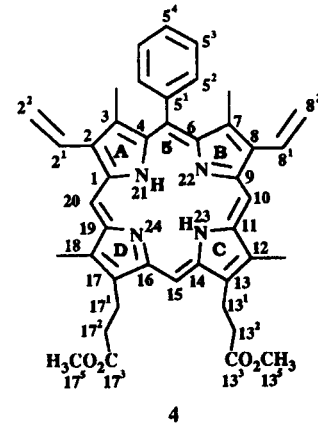
1



2



3



4

This paper is concerned with the use of a combination of one- and two-dimensional nOe experiments and theoretical calculated data by molecular modeling to determine the most favorable conformations of compounds **1-4**.

We have focused our attention on the deviation of planarity of the porphyrin macrocycle, the spatial disposition of phenyl substituents and transannular separation between hydrogen atoms attached at N-21 and N-23.

Results and Discussion.

Spectral Analysis.

The complete assignment of ^1H signals was performed by comparison with related compounds and 1D nOe difference experiments [5].

The 1D nOe and 2D NOESY methods provide a useful tool to determine connections between nuclei through space independently of bonding considerations [12].

The 2D NOESY spectra of porphyrins **1-4** provided information about the most favorable conformations of these compounds. With the aim of observing direct nOe enhancements between spatially related protons, we employed 1D nOe difference experiments.

Table 1

^1H NMR Signals (ppm) of Compound **1** Assigned by 1D nOe Difference Experiments

Percent enhancements of intensity are given in parentheses

Irradiated Signal	Enhanced Signal	Interacting Protons
2.54	3.07 (3.6)	$C(13^2)$-H, $C(13^1)$-H
	3.55 (2.1)	$C(13^2)$-H, C(12)-CH_3
	8.19 (4.3)	$C(13^2)$-H, $C(15^2)$-H
3.07	2.54 (5.4)	$C(13^1)$-H, $C(13^2)$-H
	3.55 (4.3)	$C(13^1)$-H, C(12)-CH_3
	8.19 (2.7)	$C(13^1)$-H, $C(15^2)$-H
3.55	2.54 (0.9)	C(12)-CH_3, $C(13^2)$-H
	3.07 (1.1)	C(12)-CH_3, $C(13^1)$-H
3.68	6.39 (1.0)	C(8)-CH_3, $C(7^2)$-H [b]
	8.19 (1.1)	C(8)-CH_3, $C(7^1)$-H
8.19	2.54 (3.3)	$C(15^2)$-H, $C(13^2)$-H
10.15	6.39 (1.8)	C(5)-H, $C(7^2)$-H [b]
	8.19 (10.5)	C(5)-H, $C(7^1)$-H

[b] ^1H *trans*.

Porphyrins **1** and **3** are characterized by a phenyl group attached at C-15. As shown in Tables 1 and 3, the intensity enhancement of the signals assigned to methylene protons of methoxycarbonylethyl groups [$C(13^1)$-H, $C(13^2)$-H, $C(17^1)$-H and $C(17^2)$-H] on irradiation at the resonant frequency of phenyl *ortho* proton [$C(15^2)$-H] suggested that the two methylene groups are located in the phenyl shielding region. This is corroborated by downfield shifts of 1.2 ppm for methylene protons [$C(13^1)$-H, $C(17^1)$-H] and 0.8 ppm for methylene protons [$C(13^2)$-H, $C(17^2)$-H] in porphyrins **1** and **3** with respect to the same atoms in porphyrins **2** and **4** (Tables 2 and 4). The observation of the corresponding cross-peaks in the NOESY spectra also corroborate the proposed structure. These facts are consistent with a noncoplanar orientation of the phenyl group and the plane of the porphyrin macrocycle.

Table 3

^1H NMR Signals (ppm) of Compound **3** Assigned by 1D nOe Difference Experiments

Percent enhancements of intensity are given in parentheses

Irradiated Signal	Enhanced Signal	Interacting Protons
2.53	3.06 (3.5)	$C(13^2)$-H, $C(13^1)$-H
	3.53 (1.7)	$C(13^2)$-H, C(12)-CH_3
	8.15 (4.6)	$C(13^2)$-H, $C(15^2)$-H
3.06	2.53 (4.0)	$C(13^1)$-H, $C(13^2)$-H
	3.53 (4.3)	$C(13^1)$-H, C(12)-CH_3
	8.15 (2.9)	$C(13^1)$-H, $C(15^2)$-H
3.53	2.53 (1.0)	C(12)-CH_3, $C(13^2)$-H
	3.06 (1.1)	C(12)-CH_3, $C(13^1)$-H
3.57	6.32 (1.3)	C(7) - CH_3, $C(8^2)$-H [b]
	8.22 (1.1)	C(7)-CH_3, $C(8^1)$-H
6.17	10.22 (1.4)	$C(8^2)$-H [a], C(10)-H
6.32	3.57 (1.7)	$C(8^2)$-H [b], C(7)-CH_3
8.15	2.53 (3.2)	$C(15^2)$-H, $C(13^2)$-H
	3.06 (2.1)	$C(15^2)$-H, $C(13^1)$-H
10.22	6.32 (2.2)	C(10)-H, $C(8^2)$-H [b]
	8.22 (15.2)	C(10)-H, $C(8^1)$-H

[a] ^1H *cis*; [b] ^1H *trans*.

Table 2

^1H NMR Signals (ppm) of Compound **2** Assigned by 1D nOe Difference Experiments

Percent enhancements of intensity are given in parentheses

Irradiated Signal	Enhanced Signal	Interacting Protons
3.30	4.40 (3.3)	$C(13^2)$-H, $C(13^1)$-H
	9.96 (1.4)	$C(13^2)$-H, C(15)-H
3.62	5.42 (1.8)	C(8)-CH_3, $C(7^2)$-H [b]
3.65	4.40 (1.1)	C(12)-CH_3, $C(13^1)$-H
4.40	3.30 (5.9)	$C(13^1)$-H, $C(13^2)$-H
	3.65 (1.0)	$C(13^1)$-H, C(12)-CH_3
	9.96 (5.9)	$C(13^1)$-H, C(15)-H
5.32	6.44 (8.1)	$C(7^2)$-H [a], $C(7^1)$-H
5.42	3.62 (2.0)	$C(7^2)$-H [b], C(8)-CH_3
6.44	7.91 (3.9)	$C(7^1)$-H, $C(5^2)$-H
7.91	6.44 (5.2)	$C(5^2)$-H, $C(7^1)$-H

[a] ^1H *cis*; [b] ^1H *trans*.

On the other hand, the finding that irradiation at the resonant frequencies of C(5)-H and C-8 methyl group in porphyrin **1** and C-7 methyl group and C(10)-H in porphyrin **3** enhances the intensity of the signals assigned to the vinyl methylene and methine protons [$C(7^1)$-H, $C(7^2)$-H for **1** and $C(8^1)$-H, $C(8^2)$-H for **3**] manifests the free rotation of vinyl group in both compounds. In a similar manner, the cross-peaks between both sets of methylene protons of methoxycarbonylethyl groups and C-12 methyl protons suggest that the spatial disposition of the propionic acid chain is not hindered by the presence of the phenyl group at C-15.

In the ^1H nmr spectrum of porphyrin **2** (Figure 1), the downfield shifts observed for vinyl methylene group (*ca.* 1 ppm) and vinyl methine proton (1.7 ppm) with respect

Table 4

^{1}H NMR Signals (ppm) of Compound **4** Assigned by 1D nOe Difference Experiments

Percent enhancements of intensity are given in parentheses

Irradiated Signal	Enhanced Signal	Interacting Protons
2.52	6.13 (0.9)	C(7)-CH_3, C(8^2)-H [b]
	8.10 (4.3)	C(7)-CH_3, C(5^2)-H
	8.15 (1.7)	C(7)-CH_3, C(8^1)-H
3.28	3.61 (0.9)	C(13^2)-H, C(12)-CH_3
	4.37 (3.0)	C(13^2)-H, C(13^1)-H
	9.91 (3.6)	C(13^2)-H, C(15)-H
3.61	4.37 (1.3)	C(12)-CH_3, C(13^1)-H
4.37	3.28 (6.1)	C(13^1)-H, C(13^2)-H
	3.61 (2.7)	C(13^1)-H, C(12)-CH_3
	9.91 (8.2)	C(13^1)-H, C(15)-H
6.13	2.52 (0.9)	C(8^2)-H [b], C(7)-CH_3
	10.25 (3.2)	C(8^2)-H [b], C(10)-H
8.10	2.52 (3.6)	C(5^2)-H, C(7)-CH_3
8.15	10.25 (1.9)	C(8^1)-H, C(10)-H
9.91	3.28 (4.7)	C(15)-H, C(13^2)-H
	4.37 (11.9)	C(15)-H, C(13^1)-H
10.25	6.13 (1.5)	C(10)-H, C(8^2)-H [b]
	8.15 (10.0)	C(10)NH, C(8^1)-H

[b] ^{1}H *trans*.

to porphyrins **1**, **3** and **4** indicate that these protons are located in the phenyl shielding region similar to the positioning of the methylene protons of methoxycarbonylethyl groups in porphyrins **1** and **3** (see Tables 1 and 3). In porphyrins **1** and **3** we can assume that the phenyl group adopts a spatially noncoplanar orientation relative to the plane of the macrocycle.

As shown in Table 2, nOe correlations were observed for C(7^1)-H and C(5^2)-H, as well as for H_{trans} of the vinyl group and C-8 methyl protons. According to the observed nOe, the vinyl group adopts a preferred conformation; free rotation is restricted by the presence of the phenyl group at C-5.

In porphyrin **4**, the C-7 methyl protons are upfield shifted in *ca.* 1.1 ppm with respect to the rest of methyl protons attached at the porphyrin macrocycle. This is due to the presence of the phenyl group at C-5 as corroborated by nOe correlations between C(5^2)-H and C-7 methyl protons (see Table 4). Therefore, we assume that the phenyl group lies spatially noncoplanar with the macrocycle as seen for porphyrins **1** and **3**.

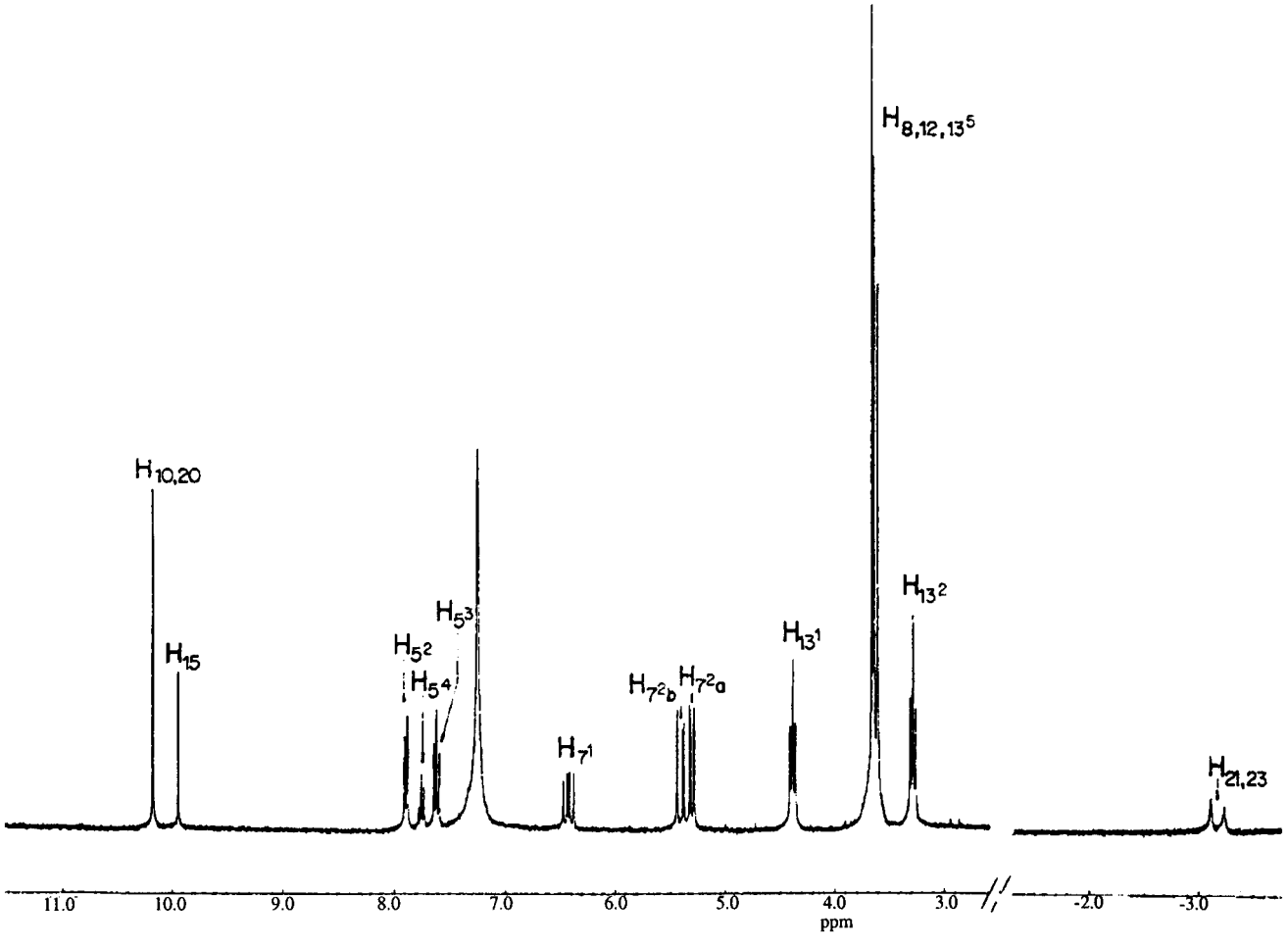


Figure 1. The ^{1}H nmr spectra of protoporphyrin **2**.

Molecular Modeling Studies.

Molecular mechanics calculations were used to investigate the energetically favored conformations of porphyrins **1-4** and compare the energy minimized 3D-structures with those suggested by the nOe nmr results. The energy-minimum conformations were calculated by full-scale geometry optimization without any constraints.

The 24-atom porphyrin core is nearly planar for porphyrins **1** and **3** (Figure 2). The individual pyrrole rings are canted with respect to each other from 0.1-3.6° (Table 5). The calculated results suggest a predominance of the conformer with a C(14)-C(15)-C(15^1)-C(15^2) torsional angle of 89.8° for porphyrin **1** and of 89.3° for porphyrin **3** (Figure 3), which is in accordance with the nOe difference effects observed on the methylene protons of methoxycarbonylethyl groups on irradiation of the phenyl C(15^2)-H protons.

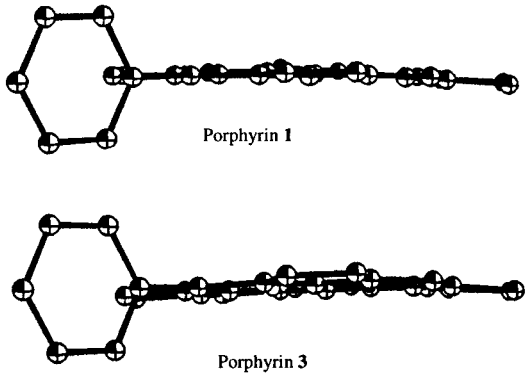


Figure 2. Optimized geometry for porphyrins **1** and **3**. All the substituents, except the phenyl group, were eliminated in order to show the spatial disposition of the macrocycle.

In order to explore the energy barriers for twisting phenyl ring with respect to the porphyrin ring plane, the dihedral angle at the covalent attachment point (C-15) was forced from 90° to 0° in 15° increments using the dihedral driver option of PCMODEL. With this option, the particular dihedral angle is fixed at a specified value, and all the other degrees of freedom are allowed to relax. The increase in energy for twisting the phenyl group 10° away from 90° is comparatively slight (ΔE~0.6 kcal/mol) for both porphyrins, **1** and **3** (Figure 4), and would be consistent with the geometrical flexibility of the methoxycarbonylethyl groups. Similar observations were performed by Monaco and Zhao [6] for *meso*-tetrakis(*N*-methylpyridyl)porphyrins. The proportion of each minimum conformation was calculated by means of the Boltzmann equation. The calculated results suggest a predominance of the conformer with a C(14)-C(15)-C(15^1)-C(15^2) torsional angle of 90° (65%) with a contribution of

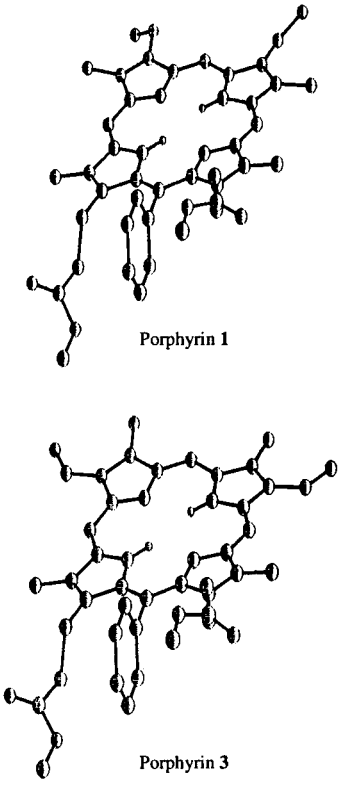


Figure 3. Spatial disposition of the phenyl group with respect to the macrocycle in the energy-minimum conformations of porphyrins **1** and **3**.

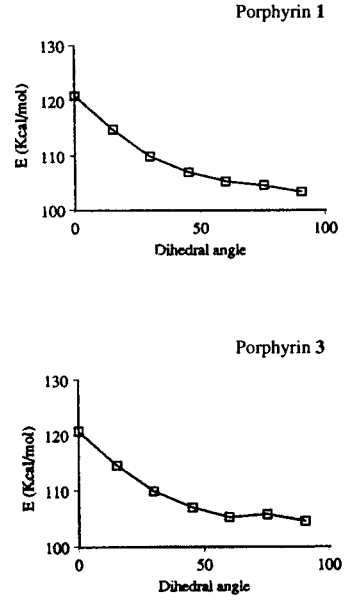


Figure 4. Optimized MMX energies for porphyrins **1** and **3** when the phenyl ring is rotated with respect to the adjacent part of the porphyrin plane from 0° to 90°.

the one of 80° (25%) and 60° (10%). In order to find the most favorable conformation of the methoxycarbonylethyl groups we computed the potential energy for torsional angle increments of 10° for rotations about the C(13)-C(13^1), C(13^1)-C(13^2) and C(13^2)-C(13^3) bonds. The results obtained indicate an *anti* conformation for the substituents of C(13^1) and C(13^2) atoms.

On the other hand, the computation of potential energy for torsional angle increments of 10° for rotation about C(7)-C(7^1) bond in porphyrin **1** and C(8)-C(8^1) bond in porphyrin **3** suggested no preferred disposition of the vinyl group in both compounds, as was inferred from the nmr results.

The presence of a phenyl group at C-5 in porphyrins **2** and **4** leads to a distorted macrocycle in both compounds, adopting nonplanar saddle conformations (Figure 5).

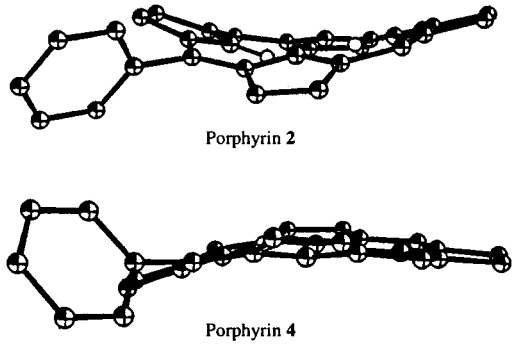


Figure 5. Optimized geometry for porphyrins **2** and **4**. All the substituents, except the phenyl group, were eliminated in order to show the spatial disposition of the macrocycle.

The individual pyrrole rings are canted with respect to each other by from 9.9-15.3° (Table 5), reflecting the distortion of the porphyrin skeleton in comparison with porphyrins **1** and **3**. The optimized geometry for both compounds shows C(5^2)-C(5^1)-C(5)-C(6) torsional angles of 64.4° for porphyrin **2** and of 67.3° for porphyrin **4** (see Figure 6). This is in accordance with the spatial disposition of the phenyl groups in *meso*-tetraphenyl porphyrin, described by Tulinsky [13]. X-ray crystallographic studies showed that this last molecule is nonplanar with angles of 61.0° and 63.1° for phenyl groups with respect to the least-squares porphyrin plane.

In the same manner as for porphyrins **1** and **3**, we have calculated the energy barriers for twisting the phenyl ring attached at C-5 in porphyrins **2** and **4**, by forcing the dihedral angle from 90° to 0° in 10° increments. The results obtained (Figure 7) show optimized geometries with dihedral angles of 70° for both porphyrins, although variations of 10° in these angles respect to 70° indicate that the energy does not vary significantly (ΔE~0.3 kcal/mol).

On the other hand, the energy increments observed for

Table 5

Dihedral Angle Calculated for Porphyrins **1-4** by Molecular Modeling

Torsion angle	1	2	3	4
N(21)-C(l)-C(20)-C(19)	3.7°	-17.0°	3.9°	13.2°
N(21)-C(4)-C(5)-C(6)	-1.8°	11.2°	-1.5°	-8.5°
N(22)-C(6)-C(5)-C(4)	-1.9°	16.8°	-3.6°	-12.9°
N(22)-C(9)-C(l0)-C(11)	4.1°	-17.9°	4.6°	13.6°
N(23)-C(11-C(10)-C(9)	0.9°	-6.5°	1.6°	3.7°
N(23)-C(14)-C(15)-C(16)	-2.6°	11.2°	-2.0°	-4.0°
N(24)-C(16)-C(15)-C(14)	-0.1°	4.4°	-0.5°	-2.3°
N(24)-C(19)-C(20)-C(1)	1.0°	-1.7°	0.3°	0.5°
C(5^2)-C(5^1)-C(5)-C(4)		-112.6°		110.3°
C(5^2)-C(5^1)-C(5)-C(6)		64.4°		-67.3°
C(15^2)-C(15^1)-C(15)-C(14)	-89.8°		89.3°	
C(15^2)-C(15^1)-C(15)-C(16)	89.7°		-90.3°	

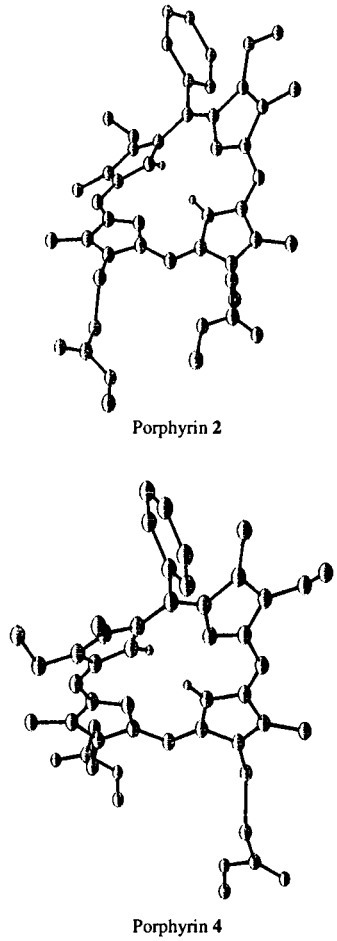


Figure 6. Spatial disposition of the phenyl group with respect to the macrocycle in the energy-minimum conformations of porphyrins **2** and **4**.

porphyrins **1** and **3** on twisting the phenyl ring 10° around the dihedral angle of the preferential conformation are of 0.6 kcal/mol. This fact could be explained by steric interaction between the phenyl and the methoxycarbonylethyl groups, which increments the energy of the conformer as the dihedral angle reduces its value to 0°. As a result of

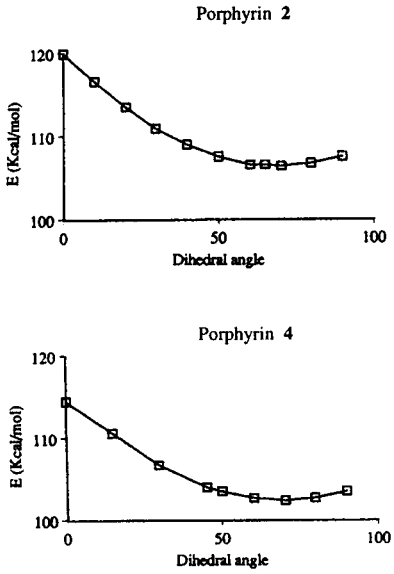


Figure 7. Optimized MMX energies for porphyrins **2** and **4** when the phenyl ring is twisted with respect to the adjacent part of the porphyrin plane from 0° to 90°.

calculations by means of the Boltzmann equation the proportions of the conformations of minor energy are 19%, 36% and 32% for the conformers with a C(5^2)-C(5^1)-C(5)-C(6) torsional angle of 80°, 70° and 60°, respectively, for porphyrin **2**. In the case of porphyrin **4** the proportions are of 24%, 37%, and 25% for the above angles, which could be explained by a minor steric hindrance of the methyl groups attached at C-3 and C-7 in comparison with the vinyl groups at the same positions in porphyrin **2**.

In order to find the preferred spatial disposition of the vinyl group we rotated the C(7)-C(7^1) bond with torsional angle increments of 10° and the results obtained showed a predominance of the conformer with a C(7^2)-C(7^1)-C(7)-C(8) torsional angle of 45.2°. This fact is responsible for the pronounced distorsion of planarity of porphyrin **2** with respect to the other three porphyrins. This observation also corroborates the nOe enhancement (5.2%) of the signal of C(7^1)-H on irradiation of the phenyl C(5^2)-H protons and is also reflected in its higher energy relative to porphyrins **1**, **3** and **4** (Table 6).

Table 6

Energies Calculated for Porphyrins **1-4** by MMX

1. E: 103.457 Kcal/$_{mol}$
2. E: 106.681 Kcal/$_{mol}$
3. E: 104.700 Kcal/$_{mol}$
4. E: 102.492 Kcal/$_{mol}$

In the same manner as for porphyrins **1** and **3**, the methoxycarbonylethyl groups adopt an *anti* conformation for the substituents of C(13^1) and C(13^2) atoms.

The transannular separations of the N-H hydrogen atoms are calculated to be 2.13, 2.13, 2.15, and 2.11 Å for the minimum-energy conformations of porphyrins **1-4**, respectively. These distances are significantly shorter than that found in octaethyl porphine (OEP: 2.36 Å) [14] and in porphine (2.41 Å) [15] but similar to those measured in mesoporphyrin IX dimethyl ester (MP-IX DME) [16].

Molecular mechanics calculations predicted the formation of hydrogen bondings (NH···N), two and one, in the preferred conformations of porphyrins **1** and **3**, respectively. Porphyrins **2** and **4**, due to their nonplanar saddle conformations are unable to present this type of bonding.

In conclusion, the results obtained by MMX force field calculations for the porphyrins **1-4** can be correlated with the nOe nmr data and should contribute to a better understanding of active site architecture in the enzyme under study.

EXPERIMENTAL

Synthetic Procedure.

Protoporphyrins **1-4** were prepared by the modified MacDonald synthesis starting from the appropriate dipyrrilmethanes as described elsewhere [5].

NMR Measurement.

The nOe difference spectra were performed on a Bruker MSL-300 spectrometer at 300.1 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane. Porphyrin spectra were obtained from dilute (9 x 10^{-3}-12 x 10^{-3} m*M*) solutions in deuteriochloroform. The nOe difference experiment was performed by presaturation of the signal and substraction of the FID of the control spectrum from the FID on irradiation. The nOe difference spectra were recorded for saturation time of 2 seconds and irradiation power levels between 30 and 36 dB below 2W depending on the selectivity required. 2D NOESY spectra were acquired at 200.1 MHz on a Bruker ACE-200 spectrometer. The relaxation delay was 2 seconds and mixing times were 800 msec with a 3% random variation. NOESY spectra were run over a spectral width of 3000 Hz in both dimensions, with 1K data point in the time domain (t_2) and 256 blocks in the evolution domain (t_1). For each of the 256 blocks, 32 transients were acquired per f_1 increment. Before the Fourier transformation, the data were processed with an exponential function in both F_1 and F_2 domains and zero filling was applied in the F_1 dimension.

Molecular Modeling.

The Allinger-Molecular mechanics methodology (Burket and Allinger, Molecular Mechanics, American Chemical Society, Washington, D. C., 1982) for theoretical calculations was used through the PCMODEL FOR WINDOWS (Serena Sofware Program, P. O. BOX 3076, Bloomington, IN, 47402-3076) on an IBM-PC-compatible computer.

The force field used in PCMODEL is MMX and is derived from MM2 force field of Allinger (N. L. Allinger, *J. Am. Chem. Soc.*, **99**, 8127 (1977)).

Acknowledgements.

This work was supported by a grant from the National Research Council (CONICET), Argentina. A. Robinsohn is grateful to CONICET for a fellowship. The authors are grateful to Dr. S. Giacopello and Mr. O. Podesta for recording the nmr spectra.

REFERENCES AND NOTES

[1] H. B. Dunford and J. S. Stillman, *Coord. Chem. Rev.*, **19**, 187 (1976).

[2] W. S. Caughey, Inorganic Biochemistry, Vol. **2**, G. L. Eichorn, ed, Elsevier Scientific Publishing Company, Amsterdam, 1973, Chapter 24.

[3] A. Brathwaite, *J. Mol. Biol.*, **106**, 229 (1976).

[4] M. A. Ator and P. R. Ortiz de Montellano, *J. Biol. Chem.*, **262**, 1542 (1987).

[5] A. E. Robinsohn and G. Y. Buldain, *J. Heterocyclic Chem.*, **32**, 1567 (1995).

[6] R. R. Monaco and M. Zhao, *Int. J. Quantum Chem.*, **46**, 701 (1993).

[7] C. J. Medforth, M. O. Senge, K. M. Smith, L. D. Sparks and J. A. Shelnutt, *J. Am. Chem. Soc.*, **114**, 9859 (1992).

[8] K. M. Barkigia, M. W. Renner, L. R. Furenlid, C. J. Medforth, K. M. Smith and J. Fajer, *J. Am. Chem. Soc.*, **115**, 3627 (1993).

[9] J. A. Shelnutt, C. J. Medforth, M. D. Berber, K. M. Barkigia and K. M. Smith, *J. Am. Chem. Soc.*, **113**, 4077 (1991).

[10] T. L. Horning, E. Fujita and J. Fajer, *J. Am. Chem. Soc.*, **108**, 323 (1986).

[11] A. Eschenmoser, *Ann. N. Y. Acad. Sci.*, **471**, 108 (1986).

[12] D. Neuhaus and M. Williamson, The Nuclear Overhauser Effect in Structural and Conformational Analysis, VCH, New York, 1989.

[13] S. J. Silvers and A. Tulinsky, *J. Am. Chem. Soc.*, **89**, 3331 (1967).

[14] J. W. Lauher and J. A. Ibers, *J. Am. Chem. Soc.*, **95**, 5148 (1973).

[15] B. M. L. Chen and A. Tulinsky, *J. Am. Chem. Soc.*, **94**, 4144 (1972).

[16] R. G. Little and J. A. Ibers, *J. Am. Chem. Soc.*, **97**, 5363 (1975).