Self-Consistent Field–Molecular Orbital (SCF–MO) Calculations and Nuclear Magnetic Resonance Measurements for Fosfomycin and Related Compounds

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Abstract □ In the present work, the mechanism of action of fosfomycin [(−)-(1*R*,2*S*)-(1,2-epoxypropyl)phosphonic acid] as an antibiotic agent is studied by "ab initio" quantum mechanical calculations and by ^{1}H, ^{13}C, and ^{31}P NMR measurements. Attention is focused on the relative charge density and chemical shift of the C(2) atom of the epoxy ring, which seems to be closely related with the activity of this antibiotic. The theoretical results suggest that the sulfhydryl addition should be preceded by a necessary anchoring of the phosphonate moiety on a positive group of the receptor.

Fosfomycin [(−)-(1*R*,2*S*)-(1,2-epoxypropyl)phosphonic acid] is a broad spectrum antibiotic whose mechanism of action is relatively well known.[1] Fosfomycin was shown to be a potent inhibitor of pyruvyltransferase (uridinephospho-*N*-acetylglucosamine-3-*O*-enolpyruvyltransferase) during the first step of bacterial cell wall biosynthesis, in which fosfomycin acts as an analogue of phosphoenolpyruvate forming an irreversible adduct with the enzyme.[2] This inhibition reaction is highly stereospecific: neither (+)*cis*-(1,2-epoxypropyl) phosphonic acid, nor the trans homologues are active.[3] The absolute configuration of fosfomycin is (1*R*,2*S*).[4] Furthermore, the ester derivatives of fosfomycin, considered hereafter, are not active.[3] Besides, the inhibition reaction is highly selective, and fosfomycin does not show any secondary action.

Earlier studies showed that inactivation of pyruvyl transferase by fosfomycin occurs through a covalent binding of the antibiotic to a negative cysteine residue in one of the active sites of the enzyme. In fact, 2*S*-L-2-(2-amino-2-carboxypropylthio)-1-hydroxypropylphosphonate was isolated after inactivation and degradation of the enzymatic complex. The reaction may be regarded as a sulfhydryl addition across the C(2)—O bond of fosfomycin (see Figure 1), with subsequent irreversible breaking and protonation of the epoxy ring.[5] To favor this addition reaction, a positive charge should be required on the C(2) atom, in spite of the anionic character of fosfomycin in solution at physiological pH. This feature was indeed predicted by semi-empirical quantum mechanical (CNDO/2) calculations carried out on an isolated fosfomycin molecule.[6]

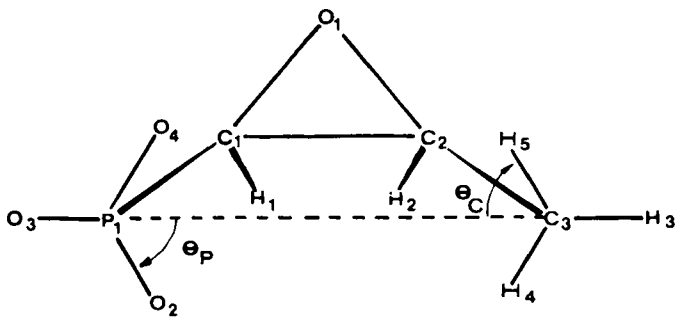

Figure 1—*Labeling of atoms and internal rotation angles in fosfomicyn.*

In the present work, charge distributions were again determined for fosfomycin, as well as for some inactive isomers of fosfomycin and their methyl ester derivatives. In the present calculations, however, the "ab initio" Gaussian procedure with the STO-4G-basis was employed. Furthermore, the charge distributions were compared with the NMR chemical shifts measured in solution. Finally, some receptor effects on the fosfomycin anion were taken into account by considering a positively charged group approaching the negative phosphonate portion of fosfomycin.

Theoretical Section

The compounds considered in the theoretical calculation were: (*1*) completely ionized fosfomycin (I-cis), ionized fosfomycin in the neighborhood of an ammonium cation (II-cis), ionized monomethyl ester of fosfomycin (III-cis), and dimethyl ester (IV-cis); and (*2*) completely ionized trans isomer of fosfomycin, (1*S*,2*S*)-(1,2-epoxypropyl)phosphonic acid (I-trans), ionized trans isomer in the neighborhood of an ammonium cation (II-trans), ionized monomethyl ester of the trans isomer (III-trans), and dimethyl ester (IV-trans). From the whole series, only the first two compounds have been shown experimentally to be active.[3]

In the calculations, the "ab initio" quantum mechanical Gaussian procedure, with minimal STO-4G basis set was used.[7] The size of the systems under study does not allow the use of a more elaborate procedure. Anyway, the calculations still considered an isolated molecule in the gas phase, so solvent effects were not taken into account explicitly in the theoretical calculation.

Since the ability of the Gaussian procedure with minimal basis set could be questioned for reproducing charge distributions, different procedures were tested in the case of fosfomycin. These calculations are given in Table I, where it can be seen that all the procedures yield similar results concerning the C(1) and C(2) atoms; that is, the charge of the C(2) atom is always more positive than that of the C(1) atom. This charge is even positive, except for the STO-4G calculation in which the negative result may be considered as fortuitous, or as a lower limit. In the following, the Gaussian STO-4G procedure will be used and considered as the "ab initio" procedure.

For the quantum mechanical calculations, the atomic coordinates of the compounds under study are needed. To determine these coordinates between the heavy atoms, two X-ray diffraction data are available: those of the monophen-

0022-3549/87/0900-0753$01.00/0

ethylammonium salt of fosfomycin, and those of the monosodium salt of the monomethyl ester of fosfomycin.[8,9]

For compounds I-cis, I-trans, II-cis, and II-trans, as well as for IV-trans, the bond length and bond angle values of the phenethylammonium salt of fosfomycin were used.[8] On the other hand, for compounds III-cis, III-trans, and IV-cis, the corresponding values of the monomethyl ester salt of fosfomycin were employed.[9]

Standard values were used for the bond lengths and bond angles of the hydrogen atoms. With these data, the preferred conformations of the two leading compounds, I-cis and I-trans, were determined by using the above-mentioned "ab initio" method. These preferred conformations were then employed in the quantum mechanical calculations of both series, cis and trans, respectively, except for compounds III-cis and III-trans.

The action of the receptor on ionized fosfomycin was mimicked by a proton and an ammonium cation conveniently located at some variable distance from the phosphorus atom. In both cases, the distance was optimized numerically. The ammonium "adduct" of fosfomycin optimized in such a way was considered as compound II.

It must be noticed here that the monoester salt of fosfomycin crystalizes into two crystallographically independent forms, A and B, having two drastically different conformations, but only slightly different bond lengths and bond angles. Both conformations were considered in two different calculations for compounds III-cis and III-trans. Finally, mean values of the bond lengths and bond angles of the monomethyl ester were used in the calculations of the dimethyl ester of fosfomycin (IV-cis).

The charge distributions of the eight compounds under study were obtained in the same calculations, but only those corresponding to the optimized configurations were retained. It may be added here that in the monomethyl ester cases, in which two forms were considered, forms A and B of fosfomycin monoester have very similar energies. So, mean values for the charges were retained. On the contrary, form B of the trans isomer has a much lower energy than form A. So, only the charges of form B were considered.

Results

Preferred Conformations—We define the internal rotation coordinates θ_c and θ_p as the $C_1—C_2—C_3—H_5$ and $C_2—C_1—P_1—O_2$ dihedral angles, respectively (see Figure 1). Conformational calculations were performed clockwise, every 30°, around these angles. The preferred conformation for the fosfomycin dianion, found using the STO-4-G approximation, was $\theta_c = 30°$ and $\theta_p = 90°$. This result differs slightly from that encountered in a previous work, that was determined by the CNDO/2 method, in which $\theta_c = 30°$ and $\theta_p = 60°$.[6]

The preferred conformation of the trans-(1,2-epoxypropyl)-phosphonate dianion was found in the same way to be $\theta_c = 60°$ and $\theta_p = 30°$. Both potential energy surfaces were relatively smooth so that some hindered rotations may be expected.

Approach of a Positive Charge—The fosfomycin approach to the receptor was mimicked in two different ways: by a proton and by an ammonium cation. These calculations were carried out at the conformations $\theta_c = 0°$ and $\theta_p = 0°$, and $\theta_c = 30°$ and $\theta_p = 90°$, respectively. In Table II, the values of the total energy and charge on the active site, C(2), as a function of the distance from the proton (or ammonium nitrogen) to the phosphorus atom are presented. It is seen that the active site, C(2), becomes more and more positive when the mimetic agent approaches the phosphonate group. In particular, the active site is found to be positive at the distance that minimizes the total energy.

Charge Distributions—The net charges obtained in the "ab initio" calculations for the I-, II-, III-, and IV-cis fosfomy-

Table I—Net Charges Calculated in Different Approximations for Fosfomycin[a]

Atom	CNDO/2		Gaussian		
	Delocalized	Localized	STO-4G	3-21G	4-31G
C(1)	0.0090	−0.2386	−0.2064	−0.4958	−0.2261
C(2)	0.1104	0.1162	−0.0218	0.0187	0.1066
O(1)	−0.2813	−0.3401	−0.2695	−0.5964	−0.6449
C(4)	−0.0006	−0.0795	−0.1980	−0.6327	−0.4715
P(1)	0.0692	1.3030	1.1974	1.9085	2.0692
H(1)	−0.1058	−0.1118	−0.0066	0.1346	0.0914
H(2)	−0.0588	−0.0643	0.0026	0.1740	0.1564
O(2)	−0.5546	−0.8523	−0.8811	−1.0385	−1.1870
O(3)	−0.5470	−0.8493	−0.8740	−1.0252	−1.1655
O(4)	−0.5493	−0.8474	−0.8884	−1.0318	−1.1834
H(3)	0.0183	0.0538	0.1057	0.2928	0.2560
H(4)	−0.0794	−0.0758	−0.0005	0.0989	0.0526
H(5)	−0.0302	−0.0139	0.0405	0.1929	0.1463

[a] At $\theta_c = 30°$ and $\theta_p = 90°$; preferred conformation found in the STO-4G.

Table II—Electric Charge on C(1) and the C(2) Active Atom and Total Energy of Fosfomycin[a]

P . . . H^+, Å	Energy, a.u.	Charge		P . . . NH_4^+, Å	Energy, a.u.	Charge	
		C(1)	C(2)			C(1)	C(2)
∞	−751.5206	−0.212	−0.023	∞	−751.5211[b]	−0.207	−0.021
1.80	−752.2547	−0.201	0.008	2.80	−808.1847	−0.206	−0.006
1.60	−752.2721	−0.211	0.006	2.50	−808.2405	−0.202	−0.001
1.40	−752.2655	−0.217	0.004	2.30	−808.2629	−0.198	0.002
—	—	—	—	2.10	−808.2454	−0.193	0.006

[a] As a function of the distance to the mimetic center (H^+ or NH_4^+) of the receptor; conformations $\theta_c = 0°$ and $\theta_p = 0°$, and $\theta_c = 30°$ and $\theta_p = 90°$, respectively. [b] The ammonium group energy (−56.2623 a.u.) is not included.

cin derivatives are given in Table III. These calculations were performed at the preferred conformation of I-cis, in which $\theta_c = 30°$ and $\theta_p = 90°$, except for the III compounds where the A and B conformations were considered. The charge distributions do not depend to a large extent on the conformation, as can be seen in Table II (when P . . . X = ∞). The distance employed in the II-cis compound was the optimum P—NH_4 = 2.30 Å.

It is seen that the "ab initio" procedure yields, in general, a charge value that is similar to the CNDO/2 one for the I-Cis fosfomycin dianion, even for the above-mentioned C(2) atom, which is now slightly negative. This negative character decreases or disappears with substitution in the II-, III-, and IV-cis compounds.

The net charges were obtained in the same way for the trans isomer derivatives of fosfomycin (I-, II-, III-, and IV-trans; Table IV). These calculations were also performed at the preferred conformation of I-trans, in which $\theta_c = 60°$ and $\theta_p = 30°$ (except for compound III-trans). The distance employed in II-trans was also P—NH_4 = 2.30 Å. It can be seen that the trans derivatives show similar charge distributions and similar behavior with the substitution as the fosfomycin derivatives. The C(2) atom is slightly less positive.

Discussion

Comparison with Nuclear Magnetic Resonance Data—The NMR spectra were taken from the literature.[10–12] Since the NMR chemical shifts may be related directly with the atomic charges, the analysis of the NMR spectra will be mainly limited to this parameter. The chemical shifts (δ in ppm) in solution of the eight compounds under study are given in Tables III and IV, together with the corresponding charge values. It must be noticed that the ammonium adducts of the theoretical calculations were replaced in the NMR measurements by the corresponding α-phenethylammonium salts.

^1H Chemical Shifts—It is seen that the H(1) signals appear at higher field than the H(2) signals in the entire cis series, in contradiction with the theoretical results. (The higher the positive charge, the higher the signal.) In contrast, the spectra of the trans isomers show an opposite behavior, in accordance with the theoretical calculations. This anomalous result for the cis series cannot be explained, as suggested in the literature,[11] by an anisotropic shielding effect exerted by the phosphonate residue. Steric effects could be responsible for this difference.

Table III—Charge Distributions and Nuclear Magnetic Resonance Chemical Shifts in (−) cis-(1,2-Epoxypropyl)phosphonate Ions (Fosfomycin) (I and II) and their Mono- and Dimethyl Esters (III and IV)[a]

Atom	I		II		III		IV	
	Charge	δ, ppm	Charge	δ, ppm	Charge	δ, ppm	Charge	δ, ppm
P(1)	+1.206	−11.70	+1.298	(−36.79)	+1.277	−14.6	+1.324	−22.14
O(2)	−0.883	—	−0.803	—	−0.744	—	−0.643	—
O(3)	−0.870	—	−0.798	—	−0.738	—	+0.450	—
O(4)	−0.874	—	−0.801	—	−0.486	—	−0.438	—
C(1)	−0.212	57.60	−0.198	55.44	−0.198	52.95	−0.184	49.00
C(2)	−0.023	57.30	+0.002	56.80	−0.001	54.32	+0.033	52.89
O(1)	−0.272	—	−0.230	—	−0.221	—	−0.165	—
H(1)	+0.005	2.76	+0.035	2.67	+0.039	2.81	+0.063	2.94
H(2)	−0.007	3.06	+0.027	3.12	+0.034	3.17	+0.074	3.12
C(3)	−0.219	16.32	−0.206	15.93	−0.198	15.04	−0.196	13.72
H(3)H(4)H(5)	+0.049	1.51	+0.062	1.52	+0.060	1.52	+0.075	1.46

[a] Conformation $\theta_c = 30°$ and $\theta_p = 90°$; ^1H at 90 MHz; I, II, III, and IV in CD_3OD; tetramethylsilane as internal reference; ^{13}C at 22.6 MHz; I and II in D_2O, III in CD_3OD, and IV in $CDCl_3$; ^{31}P at 32.3 MHz; I in D_2O, II and III in CD_3OD, and IV in $CDCl_3$.

Table IV—Charge Distributions and Nuclear Magnetic Resonance Chemical Shifts in trans-1,2-(Epoxypropyl)phosphonate Ions (I and II) and Mono- and Dimethyl Esters (III and IV)[a]

Atom	I		II		III		IV	
	Charge	δ, ppm	Charge	δ, ppm	Charge	δ, ppm	Charge	δ, ppm
P(1)	+1.196	−13.56	+1.294	−13.60	+1.277	−16.78	−1.327	−20.80
O(2)	−0.887	—	−0.806	—	−0.749	—	−0.616	—
O(3)	−0.883	—	−0.802	—	−0.728	—	−0.428	—
O(4)	−0.874	—	−0.800	—	−0.483	—	−0.427	—
C(1)	−0.201	58.95	−0.195	56.92	−0.193	55.48	−0.186	50.57
C(2)	−0.030	57.03	−0.002	56.88	−0.003	56.30	+0.038	52.79
O(1)	−0.270	—	−0.228	—	−0.225	—	−0.169	—
H(1)	+0.001	2.55	+0.034	2.57	+0.037	2.58	+0.052	2.90
H(2)	+0.035	3.18	+0.054	3.15	+0.044	3.15	+0.088	3.28
C(3)	−0.186	19.83	−0.188	19.53	−0.188	19.46	−0.191	17.41
H(3)H(4)H(5)	+0.033	1.33	+0.051	1.32	0.053	1.36	0.075	1.35

[a] Conformation $\theta_c = 60°$ and $\theta_p = 30°$; ^1H at 90 MHz; I, II, III, and IV in CD_3OD; tetramethylsilane as internal reference; ^{13}C at 20 MHz; I, II, and III in D_2O, IV in $CDCl_3$; ^{31}P at 32.3 MHz; I in D_2O, II and III in CD_3OD, and IV in $CDCl_3$.

The H(3) signals appear at a lower field in the cis than in the trans series, in accordance with the theoretical results. Again, steric effects due to the negative phosphanate moiety, which is closer to the methyl group in the cis series, are responsible for a larger shift of the electronic cloud from the H(3) to the C(3) atoms.

^{13}C Chemical Shifts—The C(2) signals appear at a lower field than the C(1) ones in the whole series. And, in accordance with the theoretical results (except for the dianions I-cis and I-trans) and to a lesser extent, the C(2) signals are at a lower field than the C(1) signals for the phenethylammonium salt (II-trans). This disagreement could be explained, in some way, by taking into account the solvent effects. Indeed, the higher the ionization of a molecule, the higher is its solvation. In the same way, the more open a molecule, the higher is its solvation. So, the chemical shifts between C(2) and C(1) appear to be reversed in the I compounds. For the same reasons, the inversion is larger in the case of the trans isomer.

The C(3) signals appear at a higher field in the cis than in the trans series, in accordance with both the theoretical results and the steric and solvent effects explained above.

Approach to the Receptor—In the present work we mainly focused our attention on the "positive" charge density on the C(2) atom of fosfomycin, which seems to be closely related with the activity of this antibiotic. In addition, since fosfomycin possesses a very negative phosphonate moiety at physiological pH, we assumed that the phosphonate residue acts as an anchoring group on the receptor. The receptor was then mimicked by a positive group approaching fosfomycin. It was found that the mimetic agent more effectively induces a positive charge on the C(2) than on C(1) atom.

We are aware that the attacking sulfhydryl residue, as well as that of an acidic proton, must be taken into account for a full understanding of the mechanism of action of fosfomycin. This work, however, seems to show that the receptor sulfhydryl attack on the C(2) atom should be preceded by the anchoring of the phosphonate group, which induces a more positive charge on C(2). The necessity of this first step seems to be confirmed by the lack of activity of the ester derivatives, where the anchoring could not occur because of steric hindrance, whenever these compounds were able to cross the bacterial cell wall.

Conclusions

As said before, a positive charge on the C(2) atom of the epoxy ring of fosfomycin is necessary for the sulphydryl addition. In this work, this C(2) atom is verified to be "positive" by using different theoretical procedures, in spite of the negative character of the whole molecule in solution at physiological pH.

Furthermore, it is shown that the anchoring of the phosphonate moiety on a positive group (which could belong to the receptor) significantly increases the "positive" nature of the C(2) atom (i.e., its reactivity). This feature suggests that the anchoring of the phosphonate moiety should be a previous and necessary step for the sulphydryl addition.

Steric effects could be responsible for the stereospecificity of fosfomycin, as well as the lack of activity of the ester compounds.

Finally, a relatively good correlation is observed between the theoretical and NMR experimental results.

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