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The role of amphotericin B amine group basicity in its antifungal action. A theoretical approach

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Abstract

The role of basicity of the amine group of amphotericin B in the molecular mechanism of antifungal activity of this antibiotic has been investigated by AM1 and MNDO quantum chemistry methods. Calculations of proton affinity of the amine group, as a measure of its basicity, for appropriate models of free amphotericin B and its *N*-alkyl derivatives were carried out. These studies were preceded by a critical examination of the usefulness and reliability of both methods to predict the proton affinities of several aliphatic amines. It has been concluded that the diminution of protonability of the substituted amine group of amphotericin B correlates with the decrease of antifungal activity of the appropriate derivatives of antibiotic. It was experimentally demonstrated (A. Czerwiński et al., J. Antibiot. 44 (1991) 979) that the introduction of additional amine groups in such a derivative restores antifungal activity of the compound. In our studies it was evidenced, using theoretical methods, that the proton affinity of this additional amine group is similar to that in free amphotericin B.

Keywords: Amphotericin B; Aminosugar; Aliphatic amines; Proton affinity of amine group; AM1; MNDO

1. Introduction

Amphotericin B (AmB) is a clinically important antifungal agent. Its mechanism of action on eukaryotic cells (including fungal and animal organisms) is based on the specific binding to the membrane located sterols [1,2] and induction of lethal changes in membrane permeability (for recent reviews, see refs. [3–6]). It has been suggested that the conformational differences between fungal ergosterol and mammalian cholesterol are responsible for the higher affinity of the drug towards the former sterol, thus allowing the use of the antibiotic as antifungal agent [7].

On the basis of structure-activity relationship studies [8,9] the hypothetical molecular model of polyene sterol complex has been proposed [10]. In this model, the hydrogen bond formed between the protonated amine group of the antibiotic as a proton donor and the oxygen atom of the hydroxyl group of sterol as a proton acceptor is essential for the formation of the complex. It has been also evidenced that the location of the protonable amine group is not restricted to its

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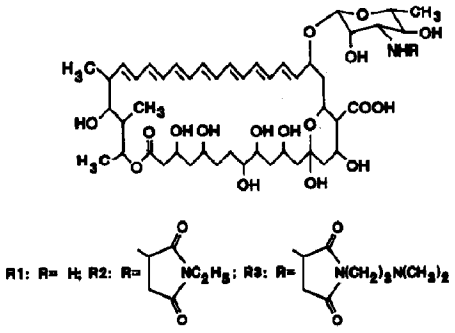

Fig. 1. Amphotericin B (R1) and its two *N*-derivatives (R2 and R3).

natural position in mycosamine moiety and can be shifted by proper introduction of substituent with the retaining of the antifungal activity [11–13].

The main goal of the present study was to evaluate the basicity (protonability) of the amine group of AmB and of its selected derivatives by a theoretical approach and to correlate the obtained results with the antifungal activity of the compounds. The appropriate AmB derivatives [13], on the basis of which the simplified models for the calculations were constructed, are presented in fig. 1. The compound R2 exhibits markedly diminished antifungal activity compared to that of the native AmB (compound R1). On the other hand, the derivative R3, bearing an additional amine group exhibits restored antifungal activity.

To make quantum chemistry calculations feasible for studied molecules three appropriate models were built (fig. 2). Models A, B, and C correspond to the compounds R1, R2, and R3 (from fig. 1), respectively.

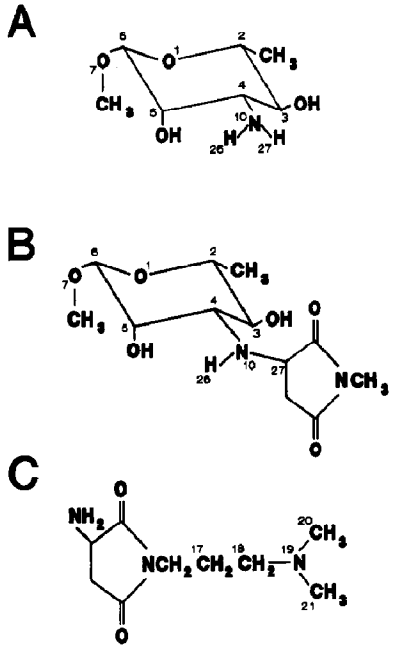

Fig. 2. Model molecules A, B, and C corresponding to compounds R1, R2, and R3 (from fig. 1), respectively.

2. Theory and methods

In order to compare the basicity of the given amine groups, proton affinities (PA) of appropriate nitrogen atoms were taken into account. A proton affinity [14] as a negative enthalpy change of the hypothetical reaction of protonation

$$B + H^+ \rightarrow BH^+ \qquad (1)$$

reliably estimates the basicity of a given species B. There is an absence (in the literature) of experimental as well as theoretically calculated proton affinity or basicity data for aminosugars and other systems that complicated. Due to this fact, the proton affinity was calculated by a quantum chemical method and it was also investigated how this proton affinity depends on conformation.

All calculations on proton affinity were carried out by means of the AM1 [15] and the MNDO [16] method. In present calculations the proton affinity was determined by employing the heat of formations according to

$$PA = \Delta H^\circ_f(B) + \Delta H^\circ_f(H^+) - \Delta H^\circ_f(BH^+), \qquad (2)$$

where $\Delta H^\circ_f(B)$ and $\Delta H^\circ_f(BH^+)$ are calculated by AM1 or MNDO heats of formation for free and protonated molecules, respectively, and $\Delta H^\circ_f(H^+)$ is the experimental value (367.2 kcal/mol) [17] of the heat of formation for H^+, AM1 and MNDO methods were chosen because they are one of the most sophisticated semiempirical quantum chemical methods, which have been

shown to reproduce the ground-state properties of a very wide variety of compounds in a satisfactory and generally economical and convenient manner [15,18,19]. It is worth stressing that due to the size of studied molecules and consumption of computer time *ab initio* calculations were not possible to perform.

There are many reports in the literature about application of the AM1 and the MNDO method to proton affinity studies [20–32]. There are even some suggestions [27–29,32] that the MNDO method is not accurate enough to allow good quality prediction of unknown PA values. It was found that the proton affinity values obtained by AM1 and PM3 [33] methods were closer to the experimental data than those calculated by MNDO. On the other hand, it should be stressed, that in comparative studies (as it is in our case) the precise values of proton affinity (compared to the experimental ones) are not as important as their actual trend within the studied set of compounds belonging to the same chemical group. If one takes into account this problem there are not many regular studies concerning this matter for different chemically consistent groups of compounds [26–29,32].

With regard to all the above mentioned remarks and doubts, both methods were tested in order to examine the applicability of these methods to PA calculation for amine groups. Testing calculations for 25 simple nonaromatic amines were performed to find a correlation between calculated and experimental values of proton affinity in the gas phase (experimental values were taken from ref. [34]). The list of investigated compounds with proton affinity calculated by AM1 and MNDO and with their experimental values of PA (PA_{exp}) [34] is presented in table 1.

The starting structures of tested compounds were built using standard values of bond length, planar angles and dihedral angles [35] [1]. The calculations for all 25 amines (free and protonated) were carried out with full geometry optimization.

[1] Structural values from a set of parameters of the MM2 program, see ref. [36].

Table 1

The list of tested compounds with proton affinity calculated by AM1 and MNDO as well as the experimental values (taken from ref. [34]) of PA in kcal/mol. The amines are divided into three groups: (compounds 1–5) primary amines containing only C, N and H atoms (line A); (compounds 6–17) primary amines containing C, N, H and F or O atoms and secondary amines containing only C, N and H atoms (line B); (18–25) tertiary amines (line C)

Compound	PA_{AM1}	PA_{MNDO}	PA_{exp}
1 CH_3NH_2	211.1	197.9	214.1
2 $CH_3CH_2NH_2$	215.0	199.7	217.0
3 i-$C_3H_7NH_2$	216.3	201.5	218.6
4 c-$C_6H_{11}NH_2$ (cyclohexylamine)	218.4	203.0	221.2
5 t-$C_5H_{11}NH_2$	221.2	203.8	222.3
6 $CF_3CH_2NH_2$	198.2	180.2	202.5
7 $CF_2HCH_2NH_2$	204.2	187.0	207.5
8 $CF_3CH_2CH_2NH_2$	204.3	187.7	210.6
9 $CFH_2CH_2NH_2$	206.9	191.3	212.3
10 $CFH_2CH_2CH_2NH_2$	210.8	195.5	217.8
11 $HOCH_2CH_2NH_2$	209.6	197.7	221.3
12 CH_3NHCH_3	212.4	198.2	220.6
13 $CH_3OCH_2CH_2NH_2$	212.8	198.4	223.3
14 $CH_3CH_2NHCH_3$	215.9	199.9	222.8
15 $CH_3CH_2NHCH_2CH_3$	219.5	201.4	225.9
16 $(n$-$C_4H_9)_2NH$	220.9	203.1	228.4
17 $(i$-$C_3H_7)_2NH$	224.1	203.9	230.2
18 $CF_3N(CH_3)_2$	192.1	170.0	193.8
19 $(CF_3CH_2)N(CH_3)_2$	201.7	183.0	215.0
20 $(CH_3)_3N$	213.5	197.2	225.1
21 $(C_2H_5)N(CH_3)_2$	217.1	199.2	227.5
22 $(C_2H_5)_2N(CH_3)$	220.6	201.5	230.0
23 $(n$-$C_3H_7)_3N$	225.0	203.1	234.0
24 $(C_2H_5)_3N$	223.7	206.0	232.3
25 $(i$-$C_3H_7)_2N(C_2H_5)$	227.1	207.3	235.3

The performed calculations indicated that the set of studied amines should be divided into three groups on account of structural differences (table 1). A good correlation was found between PA_{AM1} or PA_{MNDO} and PA_{exp} values of proton affinity within each subset of amines (figs. 3, 4). The following regression equations were obtained for the above mentioned subgroups of compounds:
for AM1 calculations (eqs. (3a)–(3c))
– Line A (compounds 1–5)

$$PA_{exp} = 0.859\,(PA_{AM1}) + 32.7, \qquad (3a)$$

with $r = 0.987$; $S = 0.610$.

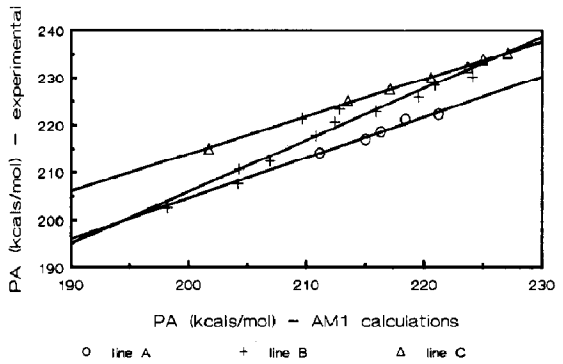

Fig. 3. Experimental proton affinity versus proton affinity calculated by AM1. Line A – primary amines containing only C, N and H atoms; Line B – primary amines containing C, N, H and F or O atoms and secondary amines containing only C, N and H atoms; Line C – tertiary amines; The definition of points and the forms of regression equations are explained in the text.

– Line B (compounds 6–17)

$$PA_{exp} = 1.094\,(PA_{AM1}) - 12.9, \qquad (3b)$$

with $r = 0.965$; $S = 2.357$.

– Line C (compounds 18–25)

$$PA_{exp} = 0.793\,(PA_{AM1}) + 55.2, \qquad (3c)$$

with $r = 0.999$; $S = 0.346$.

For MNDO calculations (eqs. (4a)–(4c))

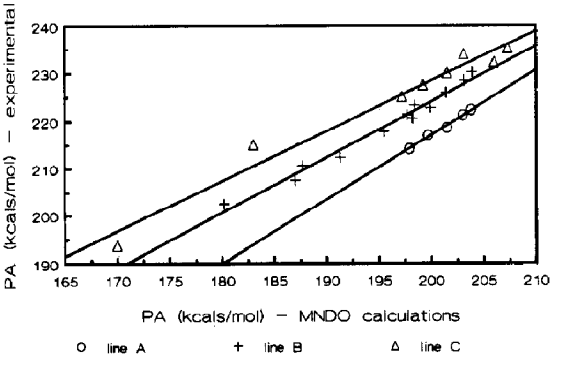

Fig. 4. Experimental proton affinity versus proton affinity calculated by MNDO. Line A – primary amines containing only C, N and H atoms; Line B – primary amines containing C, N, H and F or O atoms and secondary amines containing only C, N and H atoms; Line C – tertiary amines; The definition of points and the forms of regression equations are explained in the text.

– Line A (compounds 1–5)

$$PA_{exp} = 1.358\,(PA_{MNDO}) - 54.6, \qquad (4a)$$

with $r = 0.995$; $S = 0.358$.

– Line B (compounds 6–17)

$$PA_{exp} = 1.170\,(PA_{MNDO}) - 10.1, \qquad (4b)$$

with $r = 0.990$; $S = 1.284$.

– Line C (compounds 18–25)

$$PA_{exp} = 1.055\,(PA_{MNDO}) + 17.4, \qquad (4c)$$

with $r = 0.984$; $S = 2.625$,

where r is the correlation coefficient and S is the standard deviation. It is noticeable that line B for the AM1 calculation superimposes with line A and that points 6 and 7 (table 1) could be used to calculate regression eq. (3a) and not (3b). Nevertheless we decided to have the same subgroups of amines to calculate regression equations for both methods. On the other hand, the use of points 6 and 7 in different regression equations ((3a) or (3b)) does not change qualitatively the relative values of PA_{calc}.

The PA values derived from the regression equations, instead of the directly calculated PA values by AM1 or MNDO, were utilized to estimate the unknown experimental values of proton affinity for studied compounds presented in fig. 2.

The unexpected differences between primary, secondary and tertiary amines demonstrate that an application of the AM1 or MNDO method (and it seems that also other semiempirical methods, i.e. the PM3 method) to calculate proton affinity should be always tested at first within a chemically consistent group (or subgroup) of compounds to which the studied molecule belongs. On the other hand, it was found, despite some suggestions in the literature, that both tested semiempirical methods are good enough for comparative calculation of proton affinity of various amines. This finding is related to observations of other authors [32].

As was mentioned in section 1 only models (and not the whole AmB and its derivatives pre-

sented in fig. 1) were used in our calculations. As one can see in fig. 2 the structure of model C omits aminosugar moiety. It was assumed, however, that the aminosugar fragment of the molecule is quite distant from the studied nitrogen atom N19 and can be neglected in the calculation of proton affinity for the amine group at N19. This reasonable assumption enabled us to simplify the structure (from about 55 to about 30 atoms) of the studied derivative, which considerably decreased the consumption of computer time.

In order to explain how the dihedral angles and position of the substituents were defined, part of our own numeration of atoms in studied molecules is shown in fig. 2. Initial structures of every molecule containing only the positions of heavy atoms (C, O and N) in the aminosugar part (model A and B) and in the succinimide part (model B and C) were taken from the X-ray structural studies [37,38]. The hydrogen atoms and other atoms missing in X-ray data necessary to build full models were located using standard values of bond length, planar angles and dihedral angles [35,36]. The geometry was optimized in each calculation. The only exception was the methyl group at C2 and O7 in model A and B (aminosugar part of the molecule) and at N29 in model C (succinimide part of the molecule) (fig. 2). The geometry of these groups was optimized in the first testing calculation and then was frozen in the next calculations as not essential for our studies.

To find the relationship between proton affinity and conformation of amine group the calculations were performed in each case for three possible conformations of this group. The conformations were determined by substituents at the nitrogen atom (according to the threefold barrier of rotation around bond C4–N10 in model A and B and around bond C18–N19 in model C). Only for model B the calculations were carried out for four conformations. This fourth conformer for model B was found during optimization of the geometry for the free molecule and was taken into account with regard to its low total energy E_{TOT}.

All calculations were carried out on the VAX 6400 computer using the MOPAC program [39].

3. Results

The calculated values of proton affinity for the free aminosugar (model A) are collected in table 2 – for AM1 calculation (upper panel) and for MNDO calculations (lower panel). Together with the PA_{AM1} and the PA_{MNDO} values, the PA_{calc} values, calculated according to regression eqs. (3) and (4), are also presented. Since the aminosugar possesses substituents containing oxygen atoms similar to the tested group of amines (described by line B in figs. 3 and 4), model A was not classified by us as a typical primary amine (described by line A in figs. 3 and 4). Therefore the values of PA derived from eqs. (3b) and (4b) were also presented to make comparison with the PA values derived from eqs. (3a) and (4a). Additionally, the total molecular energy (E_{TOT}) calculated by AM1 or MNDO for the unprotonated form of each conformer (relative to the most stable conformer) is given in the third numerical column of table 2. Net atomic charges of the unprotonated and protonated nitrogen atom are also presented. In the case of model A there are no significant energetical differences between E_{TOT} as well as between PA_{calc} within the set of conformers (table 2), except for conformer 3. Conformer 3 is less stable by about a few kcal/mol compared to conformers 1 and 2. This fact is observed for AM1 as well as for MNDO calculations. Thus, it can be assumed that the populations of all three conformers are not equal. On the other hand, since the amine group is unsubstituted, we can assume free rotation of the amine group so that the protonation occurs for the more stable conformers 1 and 2.

The proton affinity PA_{AM1} (upper panel) and PA_{MNDO} (lower panel), as well as PA_{calc}, E_{TOT}, Q_A, and Q_A^+ for four conformers of the substituted aminosugar (model B) are given in table 3. The fourth conformer was added because of its low total energy for a free form. In this case the differences between proton affinity for each conformer increase to about 10 kcal/mol, which is observed for the MNDO calculations. A similar difference (about 8 kcal/mol for MNDO and 6 kcal/mol for AM1) is present between the total molecular energy of neutral (unprotonated) forms

of a given conformation. Once again conformer 3 is less stable compared to others. It means that the neutral molecule in conformation 4 (with $\omega_1 \approx -120°$, $\omega_2 \approx 120°$) or in conformation 1 (with $\omega_1 \approx -60°$, $\omega_2 \approx 180°$) is the most stable. It can be expected that mainly these conformers are protonated with a proton affinity $PA_{calc} \approx 222$ kcals/mol (for AM1) and $PA_{calc} \approx 213$ kcal/mol (for MNDO). This is ≈ 6–7 kcal/mol (upper panel, in the case of eq. (3b)) or ≈ 16–17 kcal/mol (in the case of eq. (4b)) lower than in unsubstituted aminosugar (model A). Only conformer 3 (with $\omega_1 \approx 180°$ and $\omega_2 \approx 60°$) has the proton affinity close to the level of free aminosugar but it is considerably less stable than conformers 1 and 4 in table 3. Therefore, its ability to protonation is quite small even if its proton affinity is close to that for free aminosugar. It follows that the ability to accept a proton by an amine group in substituted aminosugar (model B) is generally lower than in free aminosugar (model A).

Table 4 presents data similar to those in table 2 and 3 for model C. The proton affinities for three conformers are almost equal and no conformation dependence of the relative total molecular energy (E_{TOT}) of the unprotonated form was observed. The proton affinity of the amine group in the model C is about 5–6 kcal/mol (for AM1) and about 13–16 kcal/mol (for MNDO) higher than in the substituted aminosugar (model B). It means that the amine group in model C is a better acceptor of the proton than that in model B.

Table 2

The values of PA for the three conformers of model A. PA_{AM1} – proton affinity calculated by AM1 (upper panel), PA_{MNDO} – proton affinity calculated by MNDO (lower panel), PA_{calc} – proton affinity calculated according to eqs. (3a) or (3b) (for AM1) and eqs. (4a) or (4b) (for MNDO), E_{TOT} – total molecular energy (relative to the most stable conformer) of the unprotonated form, Q_A and Q_A^+ – net atomic charge (au) of the nitrogen atom before and after protonation. The values of PA and E are given in kcal/mol. The definition of the dihedral angles $\omega_1 \equiv$ H26–N10–C4–C3, $\omega_2 \equiv$ H27–N10–C4–C3, $\omega_3 \equiv$ H28–N10–C4–C3. The values of dihedral angles ω_i are given for the free and protonated form of the molecule

Conformation at N10 free/protonated	PA_{AM1}	PA_{calc} eqs. (3a)/(3b)	E_{TOT}	Q_A	Q_A^+
1 ω_1 = 176.5°/ 169.4°	220.6	222.2/228.4	0.0	−0.332	−0.056
ω_2 = −64.0°/ −71.1°					
ω_3 = / 48.3°					
2 ω_1 = 48.7°/ 50.7°	221.1	222.6/229.0	+0.3	−0.326	−0.055
ω_2 = −71.8°/ −68.8°					
ω_3 = / 171.9°					
3 ω_1 = 167.3°/ 172.1°	226.4	227.2/234.8	+5.8	−0.330	−0.055
ω_2 = 45.6°/ 50.9°					
ω_3 = / −68.4°					
Conformation at N10 free/protonated	**PA_{MNDO}**	**PA_{calc} eqs. (4a)/(4b)**	**E_{TOT}**	**Q_A**	**Q_A^+**
1 ω_1 = 175.2°/ −173.7°	204.5	223.1/229.3	0.0	−0.277	0.033
ω_2 = −67.6°/ −53.1°					
ω_3 = / 67.4°					
2 ω_1 = 64.2°/ 67.0°	205.3	224.1/230.3	+1.4	−0.274	0.033
ω_2 = −53.4°/ −53.7°					
ω_3 = / −174.2°					
3 ω_1 = −176.8°/ −171.7°	206.9	226.3/232.1	+3.1	−0.257	0.034
ω_2 = 68.7°/ 69.3°					
ω_3 = / −50.6°					

4. Discussion

The results obtained for three examined model compounds correlate well with the antifungal activity of the corresponding amphotericin B and its two derivatives [13]. These findings further support the hypothetical molecular model of polyene–sterol interaction which interprets the mechanism of biological activity of these compounds [10].

The basicity of the additional amine group in the studied derivative (model C) (fig. 2, atom N19) is comparable with that of the free mycosamine (model A) (fig. 2, atom N10) and greater than that of the amine group of aminosugar moiety in the model B (fig. 2, atom N10).

The results of our calculations indicate that the decrease of antifungal activity of derivative R2 (fig. 1) of AmB can be explained by the diminution of protonability of the nitrogen atom of mycosamine moiety. On the other hand, the restoration of antifungal activity in the antibiotic derivative R3 (fig. 1) bearing an additional amine group is due to the protonability of this group comparable to that of free amine group of mycosamine moiety in AmB.

It was found that calculated values of proton affinity of amine group of aminosugar moiety (fig.

Table 3

The values of PA for the four conformers of model B. PA_{AM1} – proton affinity calculated by AM1 (upper panel), PA_{MNDO} – proton affinity calculated by MNDO (lower panel), PA_{calc} – proton affinity calculated according to eq. (3b) (form AM1) and eq. (4b) (for MNDO), E_{TOT} – total molecular energy (relative to the most stable conformer) of the unprotonated form, Q_A and Q_A^+ – net atomic charge (au) of nitrogen atom before and after protonation. The values of PA and E are given in kcal/mol. The definition of the dihedral angles $\omega_1 \equiv$ H26–N10–C4–C3, $\omega_2 \equiv$ H27–N10–C4–C3, $\omega_3 \equiv$ H41–N10–C4–C3. The values of the dihedral angles ω_i are given for free and protonated form of the molecule

Conformation at N10 free/protonated	PA_{AM1}	PA_{calc} eq. (3b)	E_{TOT}	Q_A	Q_A^+
1 ω_1 = −71.3°/ −72.9°	215.1	222.4	0.0	−0.285	−0.012
ω_2 = 165.0°/ 166.7°					
ω_3 = / 44.4°					
2 ω_1 = 60.9°/ 60.5°	213.9	221.1	+1.5	−0.267	−0.007
ω_2 = −65.8°/ −61.2°					
ω_3 = / 177.9°					
3 ω_1 = 175.0°/ 175.0°	217.4	224.9	+5.9	−0.270	−0.014
ω_2 = 52.1°/ 55.4°					
ω_3 = / −68.1°					
4 ω_1 = −99.6°/ −149.1°	214.9	222.2	+0.9	−0.336	−0.007
ω_2 = 124.2°/ 88.1°					
ω_3 = / −32.3°					
Conformation at N10 free/protonated	**PA_{MNDO}**	**PA_{calc} eq. (4b)**	**E_{TOT}**	**Q_A**	**Q_A^+**
1 ω_1 = −65.8°/ −61.6°	190.8	213.3	+0.1	−0.334	−0.002
ω_2 = 166.3°/ 174.7°					
ω_3 = / 51.8°					
2 ω_1 = 80.4°/ 67.7°	192.4	215.2	+2.3	−0.342	−0.002
ω_2 = −52.5°/ −52.4°					
ω_3 = / 178.0°					
3 ω_1 = 175.0°/ −167.3°	199.6	223.6	+8.1	−0.345	−0.004
ω_2 = 45.0°/ 71.6°					
ω_3 = / −55.6°					
4 ω_1 = −115.0°/ −171.1°	190.6	213.1	0.0	−0.343	−0.006
ω_2 = 113.5°/ 67.8°					
ω_3 = / −59.4°					

2, model A and B, atom N10) depend on conformation of the amine group. It is obvious that proton affinity can be modified by intramolecular interactions. However, it is worth stressing that the introduction of bulky substituent enhances the steric effect (fig. 2, model B). This sterical crowding is the reason of the differences of proton affinity for various conformations which can be as high as 10 kcal/mol and disfavors protonation of certain conformers.

Another interesting fact is worth noting, i.e. the proton affinity of the primary nitrogen atom in aminosugar (model A), which is comparable (or even 1–2 kcal/mol higher in the case of MNDO calculations) to the tertiary nitrogen atom in the aliphatic part of mode C (when eqs. (3b) and (4b) are used). When eq. (3a) or (4a) is used to calculate PA_{calc} for unsubstituted aminosugar, then proton affinity for the primary nitrogen atom in aminosugar is lower than for the tertiary nitrogen atom in model C. Unfortunately, there are no experimental and theoretical data of proton affinity for aminosugars and because of that it is difficult to say which equations ((3a), (4a) or (3b), (4b)) are more correct for the calculation of proton affinity within this group of compounds.

Some further qualitative conclusions may also be drawn by analyzing the values of net atomic charges Q_A (tables 2, 3, and 4). The tertiary nitrogen atom of the amine group in the model C has the most negative charge compared to the amine groups in model A and B. The values of the atomic charge for nitrogen atoms (tables 2–4) increase in order: tertiary < secondary < primary nitrogen atom. This fact is observed for the MNDO calculations and agrees with the other observations and may be related to induction effects of substituents, i.e. increased electron re-

Table 4

The values of proton affinity for the three conformers of model C. PA_{AM1} – proton affinity calculated by AM1 (upper panel), PA_{MNDO} – proton affinity calculated by MNDO (lower panel), PA_{calc} – proton affinity calculated according to eq. (3c) (for AM1) and eq. (4c) (for MNDO), E_{TOT} – total molecular energy (relative to the most stable conformer) of unprotonated form, Q_A and Q_A^+ – net atomic charge (au) of the nitrogen atom before and after protonation. The values of PA and E are given in kcal/mol. The definition of the dihedral angles $\omega_1 \equiv$ H20–N19–C18–C17, $\omega_2 \equiv$ H21–N19–C18–C17, $\omega_3 \equiv$ H32–N19–C18–C17. The values of dihedral angles ω_i are given for free and protonated form of the molecule

Conformation at N19 free/protonated	PA_{AM1}	PA_{calc} eq. (3c)	E_{TOT}	Q_A	Q_A^+
1 ω_1 = 168.7°/ 165.9°	218.1	228.1	+1.6	−0.262	−0.001
ω_2 = −62.1°/ −70.6°					
ω_3 = / 47.8°					
2 ω_1 = −63.4°/ −61.0°	215.6	226.2	0.0	−0.263	0.002
ω_2 = 68.4°/ 64.0°					
ω_3 = /−178.5°					
3 ω_1 = 65.6°/ 65.2°	218.0	228.0	+1.6	−0.262	0.000
ω_2 = −162.6°/−171.2°					
ω_3 = / −53.3°					

Conformation at N19 free/protonated	PA_{MNDO}	PA_{calc} eq. (4c)	E_{TOT}	Q_A	Q_A^+
1 ω_1 = 138.6°/ 156.5°	200.3	228.7	0.0	−0.437	−0.102
ω_2 = −79.8°/ −75.6°					
ω_3 = / 41.3°					
2 ω_1 = −72.6°/ −64.0°	199.8	228.1	+0.2	−0.439	−0.101
ω_2 = 72.6°/ 67.9°					
ω_3 = /−178.1°					
3 ω_1 = 58.5°/ 67.6°	201.3	229.7	+1.0	−0.425	−0.102
ω_2 = −162.6°/−164.4°					
ω_3 = / −49.9°					

alizing ability of alkyl substituents [41–44]. Completely different results were obtained for the AM1 calculations. In this case the most negative charge for the nitrogen atom is in model A primary amine group), then in model B (secondary amine group), and then in model C (tertiary amine group). These results cannot be rationally interpreted and it seems to us that in some cases the AM1 method may give an incorrect distribution of net atomic charge – e.g. similar discrepancies were observed by other authors [44].

One should keep in mind that our calculations of proton affinity refer to the gas phase and not to the environmental conditions (lipid cell membrane) in which the antibiotic acts. However, our results are in good agreement with biological data. This good correlation between calculated and experimental data, pointing to the essential role of the amine group in antifungal action of AmB, may indicate that the influence of the lipid environment does not change essentially the relative basicity of amine groups in the examined compounds. Nevertheless further theoretical investigation with simulation of real biologically conditions would be also useful. Additionally, these studies would take into account the influence of environment and possible mediation of water molecules in the interaction between sterol and amphotericin B – according to what was suggested previously [10].

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