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# Comparative theoretical study of the conformations of amphotericin methyl ester and amphotericin B polar heads in the presence of water

S. Meddeb, J. Bergès, J. Caillet and J. Langlet

*CNRS, No. 271, Dynamique des interactions moléculaires, Université Pierre et Marie Curie, Paris (France)*

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Amphotericin methyl ester (AmE) is an interesting derivative of amphotericin B (AmB) because of its enhancement of selectivity against the fungicells. Both AmB and AmE molecules differ by the structure of their polar heads. This work deals with a theoretical study of conformations of the polar head of AmE in the presence of hydration water molecules. The results will be compared with our previous work concerning AmB.

## Introduction

Amphotericin B (AmB) is one of the most powerful antifungal polyene antibiotics and is still intensively studied (for instance see Refs. 1 and 9 and references cited herein). The mode of action of AmB at molecular level is not completely elucidated, in particular complexation with sterols and with itself is still in question [10–16]. Thus the importance of the structure of the polar head with regard to these interactions was discussed, and it has appeared important to study experimentally other derivatives [14–16]. Amphotericin methyl ester (AmE) was one of the most promising substituted molecule because of its enhancement of selectivity against ergosterol.

Let us recall that, in a preliminary work [17], we have performed a series of conformational investigations of the zwitterionic form of AmB in both isolated and hydrated states. In this present work, we present a similar theoretical study of the conformation of the polar head of amphotericin methyl ester (AmE). We will thus discuss our results and compare them with the ones that we have obtained on AmB.

Correspondence to: J. Langlet, CNRS, No. 271, Dynamique des interactions moléculaires, Université Pierre et Marie Curie, Tour 22, 4 Place Jussieu, 75252 Paris Cedex 05, France.

## Method

Both intra and intermolecular energies have been calculated simultaneously in the framework of the SIBFA method (sum of interactions between fragments computed ab initio) [18–20].

### *(1) Intermolecular energy [18,20]*

Following an additive procedure, the intermolecular energy is written as a sum of five contributions:

$$E_{\text{Inter}} = E_{\text{El}} + E_{\text{Pol}} + E_{\text{Rep}} + E_{\text{Disp}} + E_{\text{CT}} \quad (1)$$

which are calculated from analytical formulae derived from perturbation theory (SAPT = symmetry adapted perturbation theory) [21].

We can point out several characteristic features:

(i) Use of a multicenter (atom and middle of bonds), multipolar (up to quadrupoles) expansion derived [22] from the Ab-initio SCF molecular function for the calculations of electrostatic and polarization components, $E_{\text{El}}$ and $E_{\text{Pol}}$, respectively.

(ii) Computation of the repulsion term, $E_{\text{Rep}}$, as a sum of 'bond-bond', 'bond-lone-pair' and 'lone pair-lone pair' interactions. Such a representation of lone pair accounts for the radial and directional dependence of repulsion term, the analytical function being of an exponential type.

(iii) Dispersion, $E_{\text{Disp}}$, is dumped to take into account overestimation of the energy at short distances.

(iv) Explicit evaluation of the charge-transfer, $E_{CT}$, contribution between lone pairs of the electron donor molecule and hydrogen atom of the electron acceptor molecules.

*(2) Intramolecular energy [19]*

In the SIBFA method, a large molecule is built out of constitutive molecular fragments separated by single bonds. In fact one calculates the variation of the conformational energy as a sum of inter-fragments interaction energies:

$$\Delta E_{\text{Intra}} = \sum_{i=1}^{N} \sum_{j=i+1}^{N} E'_{\text{Inter}}(i, j) \qquad (2)$$

where $N$ is now the number of fragments.

$E'_{\text{Inter}}$ is calculated as a sum of the four first contributions given in Eqn. 2, plus a term denoted $E_{\text{Tor}}$ which is a transferable torsional energy contribution, calibrated for elementary rotations around single bonds (for more details concerning this method see Refs. 18–20).

*(3) Hydration energy*

As an evaluation of the solvent effect, we have only taken into consideration 'hydration water' molecules, i.e., the ones which are very close to the solute and thus interact very strongly with it. In order to estimate the 'hydration energy' ($\Delta E_{\text{Hydra}}$), it may be supposed that each water–solute interaction ($E_{\text{W-S}}$) replaces a water–water interaction ($E_{\text{W-W}}$), $N_{\text{W}}$ being the number of 'hydration water' molecules; we get

$$\Delta E_{\text{Hydra}} = E_{\text{Inter}} - N_{\text{W}} E_{\text{W-W}} \qquad (3)$$

We have used the value of $E_{\text{W-W}}$ of 5.4 kcal/mol calculated within the SIBFA method.

We are conscious that $\Delta E_{\text{Hydra}}$ only represents part of the total solvation energy in water, but such a study should give an eventual insight into possible intramolecular conformational changes due to these strong water–solute interactions.

## Results

*(A) AmE in the isolated state*

In this present study, as in our previous work, all bond lengths and bond angles were fixed to the values obtained from an X-ray study of *N*-iodoacetyl AmB [24] and standard values are taken for the methyl groups. Because of the presence of a conjugated double bond system, we consider that the heptaenic macrolactone ring remains rigid and therefore independent of the surrounding medium. Perun and Egan [25] have confirmed in the case of erythromycin, another

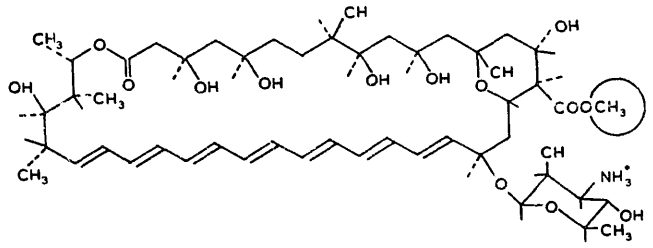

Fig. 1. Amphotericin B (AmB)/Amphotericin methyl ester (AmE).

macrolide antibiotic, that the conformation of the macrolactone ring does not change when it is compared to crystal state. Furthermore, from a chemical point of view, AmE and AmB only differ by their polar head. Hence we have still kept this part of the molecule within the conformation established in the crystal, and we have been interested only by the flexible polar head.

The conformational energy is expressed as a function of the nine variable dihedral angles $\alpha_i$ ($i = 1, 9$) defined in Fig. 2.

*(1) Optimisation processes*

In order to explore the conformational space of AmE, we first performed a direct optimisation process involving simultaneously the nine dihedral angles $\alpha_i$: the geometrical arrangement ($A_0$) determined in the crystal has been chosen as a guessing point. Then, as a second alternative we have calculated different energy submaps $E = f(\alpha_i, \alpha_j)$ keeping all other angles frozen at the value that they have in $A_0$.

*(a) Optimisation of $A_0$ conformation.* It results in a conformation denoted $A_1$ with a 41 kcal/mol stabilisation mainly proceeding from a simultaneous change in $\alpha_2$ and $\alpha_3$ dihedral angles.

*(b) Conformational two dimensions sub-maps studies.* as a first step, it has been performed a rough exploration of the different energy sub-map $E = f(\alpha_1, \alpha_j)$. The choice of the two angles $\alpha_i$ and $\alpha_j$ proceeds on their mutual dependency. The two dihedral angles

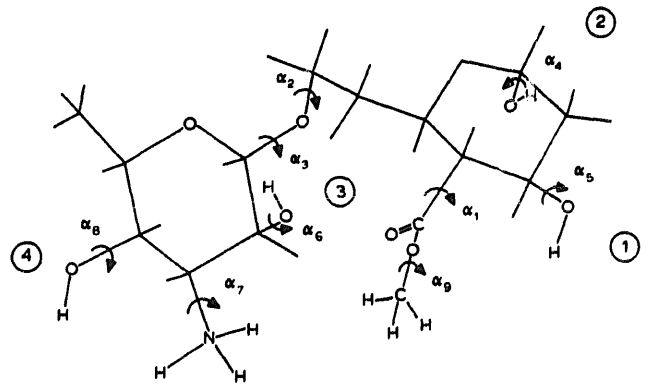

Fig. 2. Polar head of AmE. The $\alpha_i$ dihedral angles are noted. The circled numbers are related to the hydroxyl groups.

TABLE I

*Variations of the intramolecular energy* $\Delta E_{Intra}$ and of its components for the isolated state of AmE

All the values are in kcal/mol.

| Conformation | $\Delta E_{Rep}$ | $\Delta E_{Disp}$ | $\Delta E_{El}$ | $\Delta iE_{Pol}$ | $\Delta E_{Tor}$ | $\Delta E_{Intra}$ |
|---|---|---|---|---|---|---|
| $A_1$ | 3.4 | 0.8 | −10.8 | 0.7 | −5.5 | −41.3 |
| $A_2$ | 4.2 | −2.1 | −53.4 | 1.2 | −3.9 | −54.1 |
| $A_3$ | 19.3 | −17.7 | −56.6 | −0.6 | −3.0 | −58.4 |
| $A_4$ | 6.6 | −2.5 | −54.9 | 1.8 | −4.3 | −53.3 |

have been varied by steps of 30 degrees. Such a study has shown that the multidimensional conformational energy space is restrained to only the three dihedral angles (namely $\alpha_1$, $\alpha_2$ and $\alpha_3$) that govern the geometrical structure of the polar head. So we have then performed a more refined analysis of this three-dimensional subspace. The eleven local minima thus obtained have been chosen as guessing points for our automatic optimization process. Finally three minima denoted $A_2$, $A_3$ and $A_4$ have been obtained (Tables I and II).

*(2) Analysis of results*

The conformation of the polar head is mainly governed by electrostatic forces (see Table I).

All our minimal conformations present an intramolecular H-bond between the hydroxyl group of the lactone ring, OH(2), and the next hydroxyl group located on the macrolide ring (Fig. 3a,b,c).

The $A_3$ conformation is the most stable one. $A_2$ and $A_4$ (which are quite isoenergetic) lie nearly 4 kcal/mol above $A_3$. These three conformations are stabilized at nearly 15 kcal/mol with regard to the 'open' $A_1$.

The structure $A_2$ is an 'open' conformation: the nearest distance between $NH_3^+$ and $COOCH_3$ groups being 5.56 Å. The hydroxyl group of the mycosamine moiety is relied by intramolecular H-bonds to both $COOCH_3$ and $NH_3^+$ groups (see Fig. 3a).

TABLE II

*Values of dihedral angles in degrees for the different conformations of the polar head of AmE and of AmB [17]*

The calculated values for the three other angles are: $\alpha_4 \cong 60°$ (180° for $A_0$ and $C_1$), $\alpha_5 \cong 170°$ (120° for $A_0$ and $C_1$), $\alpha_6 \cong 60°$ (180° for $A_1$).

| Conformation | $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_6$ | $\alpha_7$ | $\alpha_8$ |
|---|---|---|---|---|---|---|
| $A_0$ | 67.7 | 272.4 | 142.1 | 360.0 | 150.0 | 360.0 |
| $A_1$ | 57.0 | 184.9 | 68.3 | 305.6 | 187.3 | 98.7 |
| $A_2$ | 271.8 | 234.4 | 75.0 | 288.6 | 46.2 | 255.8 |
| $A_3$ | 218.1 | 287.8 | 94.5 | 331.5 | 319.8 | 82.8 |
| $A_4$ | 254.4 | 257.3 | 271.4 | 292.7 | 283.9 | 246.7 |
| $C_1$ | 202.7 | 285.7 | 83.5 | 220.0 | 177.3 | 360.0 |

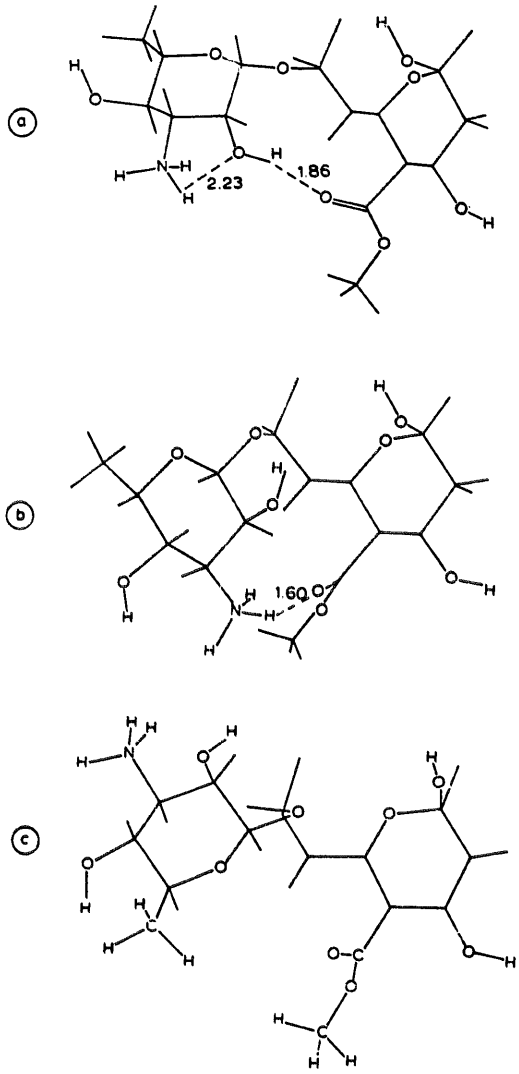


Fig. 3. Most stable conformations of polar head of AmE in the isolated state (a) $A_2$, (b) $A_3$, (c) $A_4$. Dashed lines represent the hydrogen bonds, their lengths are in Å.

The structure $A_3$ is a folded one. The folding of this structure is ensured by a strong intramolecular H-bond ($d$ = 1.6 Å) between one hydrogen of $NH_3^+$ group and one oxygen of $COOCH_3$ group (see Fig. 3b).

The $A_4$ conformation stands out against the other ones by a 180° variation of the dihedral angle $\alpha_5$. The $NH_3^+$ group is pushed at the opposite side of the $COOCH_3$ group (see Fig. 3). It does not appear any intramolecular H-bond.

*(3) Comparison between AmB and AmE conformations*

The AmE most stable conformation is rather similar to $C_1$(AmB) one: The main geometrical parameters (namely $\alpha_1$, $\alpha_2$, and $\alpha_3$ dihedral angles) are practically identical.

In AmB, the stabilization of $C_1$ is due to an array of two intramolecular H bonds one of them connecting

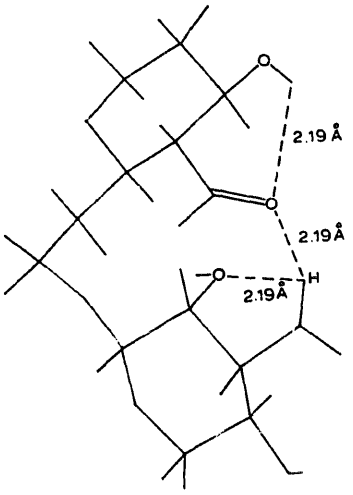


Fig. 4. Most stable conformations of polar head of AmB ($C_1$) [17]. Dashed lines represent the hydrogen bonds, their lengths are in Å.

the lactone ring and the sugar moiety. Furthermore it has been noticed that only a restricted area is allowed in the $\alpha_1$-$\alpha_3$ conformational subspace of AmB. It resulted in one unique minimum th location of which is slightly shifted when varying the $\alpha_1$ dihedral angle. Open conformations similar to $A_2$ or structure like $A_4$ (with a 180° rotation of the sugar moiety) have not been obtained. In fact the freedom of the amino sugar moiety to rotate is hindered in zwitterionic AmB by electrostatic interactions between $NH_3^+$ and $COO^-$ groups. Such a limitation is less drastic in AmE.

*(B) AmE in the hydrated state*

Some water molecules have been set in the vicinity of hydration sites, namely $NH_3^+$, $COOCH_3$ and OH groups. Saturation (in sense of hydration water molecules) occurs with nine water molecules. Optimization of both intra and intermolecular energies has been performed. An analysis of the interaction energy between each water molecule and the polar head of AmE has shown that some water molecules interact with the solute by less than 5.4 kcal/mol (water dimer energy), they have not been taken into account since they do not correspond to our definition of hydration water. So only 6 (and 7 for the crystal-like structure) water molecules have beèn considered.

TABLE III

*Variations of the intramolecular and hydration energies for the solvated molecule*

All the values are in kcal/mol.

| Conformation | $\Delta E_{Intra}$ | $\Delta E_{Hydra}$ | $\Delta E_{Tot}$ |
|---|---|---|---|
| $A'_0$ | −22.6 | −53.8 | −75.8 |
| $A'_2$ | −53.4 (+0.7) | −22.8 | −76.2 |
| $A'_3$ | −50.0 (+8.4) | −40.3 | −90.3 |
| $A'_4$ | −48.1 (5.2) | −38.0 | −86.1 |

Intra and intermolecular energies are listed in Table III.

The crystal-like structure $A_0$ is taken as zero energy conformation. The values of the different dihedral angles calculated after our full optimization process are collected in Table IV. Now in our nomenclature $A'_i$ (with $i = 0 \ldots 4$) indicates a hydrated structure obtained by optimization of $A_i$ structure surrounded by hydration water molecules.

*(1) Analysis of our results*

*(a) For the conformations of the polar head.* As shown in Table III, the $A'_3$ hydrated structure which is the most stable one lies 5 kcal/mol below $A'_4$. Now $A'_2$

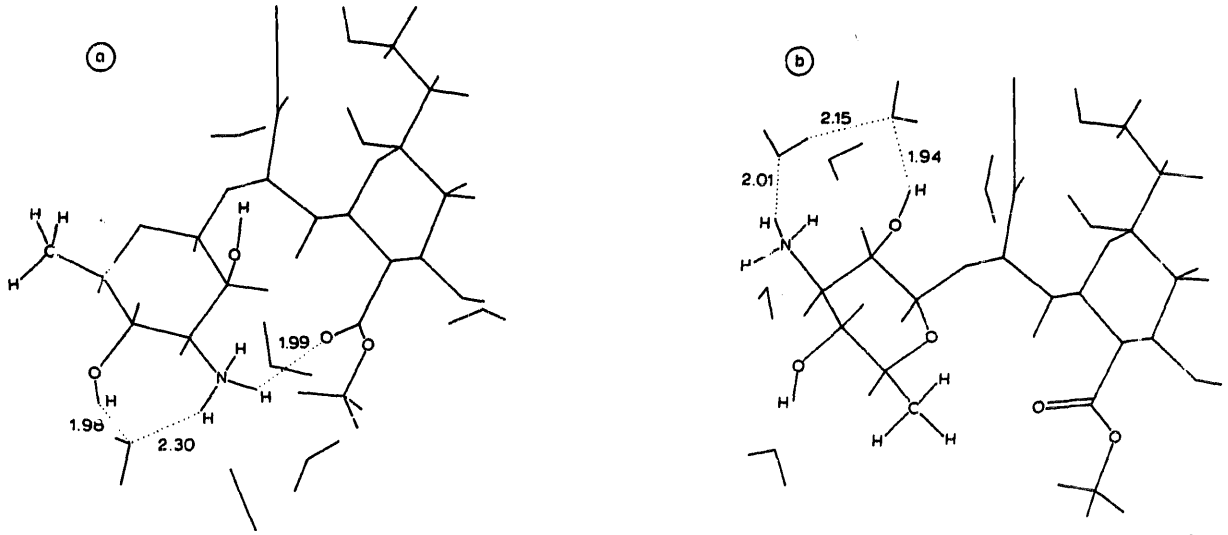


Fig. 5. Most stable structures of polar head of AmE in the hydrated state (a0 $A'_3$, (b) $A'_4$. Dashed lines represent some hydrogen bonds, their lengths are in Å.

TABLE IV

*Values of dihedral angles for the different conformations of the solvated polar head of AmE*

The calculated values for the two other angles are: $\alpha_4 \cong 65°$ (178° for $A'_0$), $\alpha_5 \cong 170°$ (142° for $A'_0$ and $A'_3$).

| Conformations | $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_6$ | $\alpha_7$ | $\alpha_8$ | $\alpha_9$ |
|---|---|---|---|---|---|---|---|
| $A'_0$ | 69.9 | 223.4 | 143.4 | 1.4 | 176.2 | 265.7 | 71.5 |
| $A'_2$ | 271.4 | 234.8 | 75.4 | 285.8 | 44.7 | 268.5 | 302.2 |
| $A'_3$ | 224.9 | 286.5 | 98.8 | 330.7 | 294.2 | 72.7 | 60.0 |
| $A'_4$ | 257.4 | 260.1 | 273.2 | 261.2 | 302.9 | 268.1 | 60.0 |

hydrated structure lies 10 kcal/mol above $A'_4$ and is isoenergetic to $A'_0$. Because of a very weak hydration energy, hydrated structure $A'_1$ is very unstable ($E \approx 30$ kcal/mol with regards to $A'_4$, so it has not to be taken into consideration.

In structure $A'_0$ the shortest distance $d_1$ between $NH_3^+$ and $COOCH_3$ has increased from 2.46 Å to 4.35 Å due to a variation of $\alpha_2$-dihedral angle. Thus conformational change stabilizes $A'_0$ by 22 kcal/mol, furthermore, as shown in Table III $A'_0$ strongly interacts with hydration water molecules. Nevertheless in spite of the gain of intramolecular energy and of the strong hydration energy, $A'_0$ remains nearly 14 kcal/mol above $A'_3$.

The presence of 'hydration' water molecules does not influence significantly the geometrical arrangement of $A_2$ conformation the intramolecular energy of which remains almost unchanged. But as $A'_1$, the $A'_2$ structure has a poor interaction energy with 'hydration' water molecules.

In both $A'_3$ and $A'_4$ structures, it may be noticed that the loss in intramolecular energy (8.4 kcal/mol and 5.2 kcal/mol, respectively) is balanced by a quite important hydration energy gain (40.3 kcal/mol and 38.0 kcal/mol, respectively). So these two structures represent the two stable hydrated complexes). Figs. 5a,b give an illustration of the position of the hydration water molecules interacting with the polar head of AmE within the geometrical arrangement $A'_3$ and $A'_4$.

TABLE V

*Variations of the intramolecular and hydration energies for the solvated state of AmB [17]*

All the values are in kcal/mol.

| Conformation | $\Delta E_{Intra}$ | $\Delta E_{Hydra}$ | $\Delta E_{Tot}$ |
|---|---|---|---|
| A″ | −17.1 | −85.7 | −103.1 |
| $C''_1$ | −29.0 (+5.4) | −76.1 | −105.1 |
| $C''_2$ | −25.8 (+2.2) | −73.4 | −99.2 |

*(b) For the hydration water molecules surrounding the polar head* Water molecules form H-bond bridges between different polar groups of the polar head of AmE.

The ether oxygen is connected to hydroxyl groups, namely OH(2) in $A'_2$ and OH(5) in $A'_4$.

One H-bridge occurs between $NH_3^+$ and OH(4) groups in $A'_3$.

Polar groups of AmE are connected via a network of two H-bonded water molecules between $NH_3^+$ and OH(3) groups in $A'_4$.

*(2) Comparison with hydrated AmB*

In our previous study of AmB we have obtained three hydrated structures (A″, $C''_1$ and $C''_2$) with comparable stabilities: (see Table V). The structure A″ has been obtained from optimization of RX structure in the presence of hydration water molecules: A″ is intermediate between the open A and the folded C structures, $C''_1$ and $C''_2$ structures which proceed from hydration of $C_1$ and are quite similar from a geometrical point of view. In AmB it has been noticed the occur-

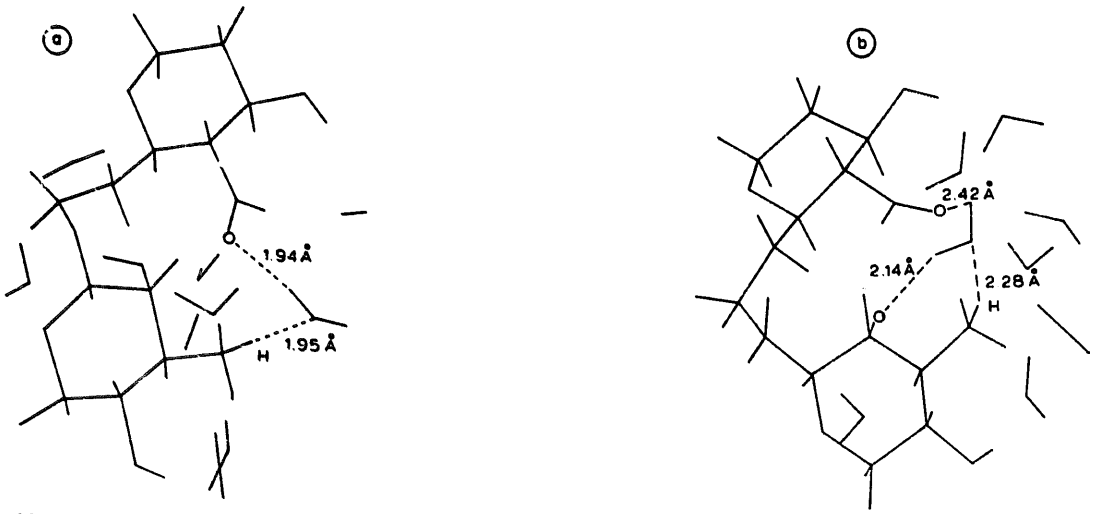


Fig. 6. Most stable structures of polar head of AmB in the hydrated state (a) $C''_1$, (b) A″ [17]. Dashed lines represent some hydrogen bonds, their lengths are in Å.

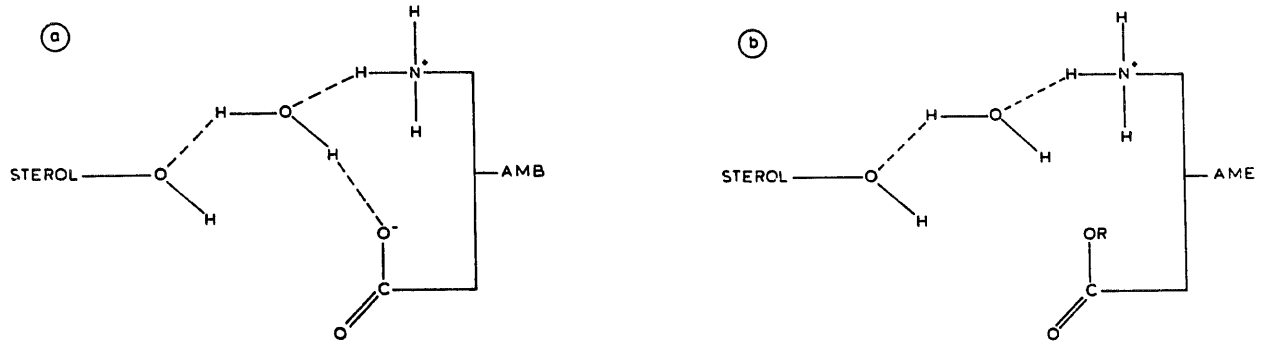


Fig. 7. Representation of the polyene-sterol hydrophilic interactions (a) AmB, (b) AmE [14].

rence of a H-bond bridged configuration involving: (a) $CO_2^-$, $NH_3^+$ and OH groups in $C_1''$ and $C_2''$, (b) $CO_2^-$ and $NH_3^+$ in A".

Such a situation is not possible in AmE because of esterification of the carboxylate group, even in the $A'_3$ structure whose geometry is similar to those of $C_1''$. But in the $A'_3$ and $A'_4$ structures (this last one being obtained only with AmE) water bridges occur between $NH_3^+$ and one OH group.

Comparison between the results displayed in Tables III and V shows that the hydration energy is more important for AmB than for AmE perhaps because of the water bridges connecting the two charged groups in AmB.

## Discussion

Before any discussion concerning the conformational difference between AmE and AmB, we want to point out that only three dihedral angles (namely $\alpha_1$, $\alpha_2$, $\alpha_3$) are fundamental for the optimization process of the conformation of both AmE and AmB. The value of these three angles determine the position of the mycosamine sugar with regards to the lactone moiety and the steric position of the $COOCH_3$ group and thus settles the formation (or not) of an intramolecular H-bond.

In the isolated state of both AmE and AmB, it has appeared a folded conformation stabilized by an intramolecular H-bond connecting $CO_2$ and $NH_3$ groups. In AmE (only) our calculations have led to two isoenergetic open conformations (denoted $A_2$ and $A_4$) one of them ($A_4$) appearing without any intramolecular H-bond. It seems that the methylation of the $CO_2$ group allows the free rotation of the amino sugar.

In the hydrated state, the hydration water molecules network is desorganized in AmE: the strong 'water bridge' connecting $CO_2$ and $NH_3^+$ in AmB has disappeared, we have only noticed one water molecule H-bond to $NH_3^+$.

The disposition is reminiscent of the representation proposed by Hervé et al. [14]: A completary (or competitive) H-bond may occur between this water molecule and the $\beta$-OH group of a sterol molecule in the complexes between amphotericin B (and its derivatives) with sterols. From our results we can reasonably ascertain that the H-bonding system relying the polar head of the antibiotic to the sterol should be stronger for AmB (group I polyenes) than for AmE (group II polyenes).

But may questions arise: Does the H-Bonding system between the antibiotic AmB or AmE and the sterols play a prominent role in the complex formation between these entities? What is the part of Van der Waals interactions? So at this level of work, it appears very interesting to obtain informations on the total interaction energy values of the complexes sterol-AmB (AmE). this will be subject of our next paper. Actually the results of such a study should probably give an insight of such a model (complex between sterol and antibiotic) to reproduce or not the selectivity of different antibiotics (group I or II) for fungal or animal cells.

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