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Proton and Sodium Cation Affinities of Harpagide: A Computational Study

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The aim of this work was to estimate the proton and sodium cation affinities of harpagide (Har), an iridoid glycoside responsible for the antiinflammatory properties of the medicinal plant *Harpagophytum*. Monte Carlo conformational searches were performed at the semiempirical AM1 level to determine the most stable conformers for harpagide and its protonated and Na^+-cationized forms. The 10 oxygen atoms of the molecule were considered as possible protonation and cationization sites. Geometry optimizations were then refined at the DFT B3LYP/6-31G* level from the geometries of the most stable conformers found. Final energetics were obtained at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G* level. The proton and sodium ion affinities of harpagide have been estimated at 223.5 and 66.0 kcal/mol, respectively. Since harpagide mainly provides $HarNa^+$ ions in electrospray experiments, the $\Delta_r G_{298}$ associated with the reaction of proton/sodium exchange between Har and methanol, $MeOHNa^+ + HarH^+ \rightarrow MeOH_2^+ + HarNa^+$ (1), has been calculated; it has been estimated to be 1.9 kcal/mol. Complexing a methanol molecule to each reagent and product of reaction 1 makes the reaction become exothermic by 1.7 kcal/mol. These values are in the limit of the accuracy of the method and do not allow us to conclude definitely whether the reaction is endo- or exothermic, but, according to these very small values, the cation exchange reaction is expected to proceed easily in the final stages of the ion desolvation process.

Introduction

Harpagophytum procumbens or *zeyheri*, also called "Windhoek seed" or "Devil's claw", is a medicinal plant from South Africa, Namibia, and Botswana. Its secondary dried, cut, tuberous roots containing harpagophyton have been included in the French pharmacopeia for about fifteen years. In phytopharmaceutical treatment, *Harpagophytum* is usually recommended to treat inflammatory processes. According to Anderson et al, *H. procumbens* acts by migration of interleukins and leucocytes to the painful and inflamed joint area and displays antiinflammatory, analgesic, and antiphlogistic properties.[1]

As a result of a number of recent studies in rats and humans, *Harpagophytum* is proposed as a complementary treatment for chronic rheumatisms, tendinitis, osteoarthrosis, and arthritis.[2−4] It acts on the muscular system. Athletes also use it to avoid or ease pains due to effort. Herbal preparations containing *Harpagophytum* with other plants are used for prevention of inflammatory processes of competition horses and in the treatment of animals suffering from lameness. Chemically, *Harpagophytum* contains a number of compounds, especially iridoid glycosides like harpagoside, harpagide (Har), and 8-*para* coumaroyl harpagide, which are assumed to be responsible for the antiinflammatory properties of the plant. Apart from the recent study by Baranska et al., there is very little information in the literature about the analysis of these compounds from vegetal medium and biological fluids.[5]

We recently developed an analytical method involving the coupling of high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) to detect and quantify harpagide in horse plasma and urine.[6] The chemical structure of harpagide is given in Figure 1.

Under electrospray ionization, a standard solution of harpagide provides much more $HarNa^+$ (sodiated harpagide) than $HarH^+$ (protonated harpagide) pseudomolecular ions, even when only vessels made of polypropylene are used (Pyrex and borosilicate vessels are well-known to provide many sodium ions). The predominance of $HarNa^+$ over $HarH^+$ ions, in the absence of added sodium salts, is a well-known phenomenon in electrospray experiments for a number of complex sugars such as N-linked carbohydrates and oligosaccharides.[7,8] Tandem mass spectrometry experiments showed that the precursor ion of $HarNa^+$ is [$HarNa^+$, MeOH]. $HarNa^+$ ions might result from proton/sodium exchange during the electrospray process of the analyte. Experiments by Amad et al. demonstrated that ions formed in solution are likely to be altered in the gas phase by the presence of molecules that are stronger gas phase bases.[9] It must be kept in mind that predominance of $HarNa^+$ ions could also be due to the presence of harpagide sodium salt in the commercial product. The first aim of this study was to estimate the proton and sodium ion affinities of harpagide and the $\Delta_r H_{298}$ associated with reaction 1, in order to determine the plausibility of the former hypothesis. $\Delta_r H_{298}(1)$ can be calculated as $\Delta_r H_{298}(1) =$

$$MeOHNa^+ + HarH^+ \rightarrow MeOH_2^+ + HarNa^+ \qquad (1)$$

$SA(MeOH) + PA(Har) - PA(MeOH) - SA(Har)$ where SA(Har) and SA(MeOH) are the sodium cation affinities of

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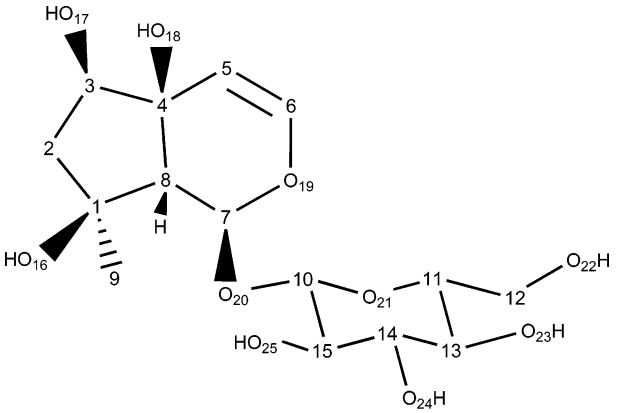

Figure 1. Chemical structure of harpagide and numbering of heavy atoms.

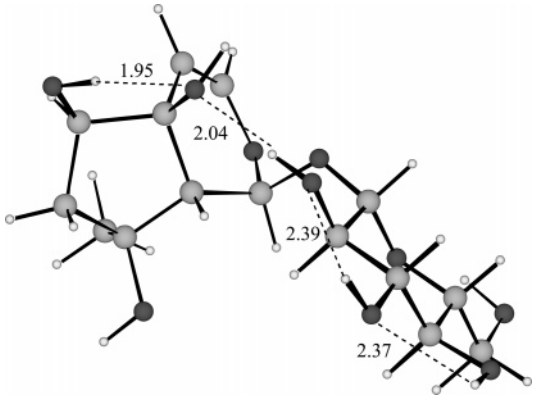

Figure 2. The most stable structure optimized for harpagide, at the B3LYP/6-31G* level. Hydrogen bond lengths (in dotted lines) are in Å.

harpagide and methanol and PA(Har) and PA(MeOH) the proton affinities of harpagide and methanol, respectively. SA(MeOH) and PA(MeOH) values are available in the literature; PA(Har) and SA(Har) have to be calculated. Since $HarNa^+$ ions were retained as precursor ions for MS/MS experiments, the second aim of this study was to obtain information about the possible structures of $HarNa^+$, in order to interpret, in further work, the collision induced mass spectra of cationized harpagide.

Computational Methods

Semiempirical Calculations. Geometry optimizations were first performed at the semiempirical AM1 level,[10,11] with the Hyperchem 7.5 package for Windows.[12] Given the large number of possible conformers of Har, $HarH^+$, and $HarNa^+$, Monte Carlo conformational searches were performed on the neutral form and on the cationized structures. Randomized values were assigned to the torsion angles that traduce the rotation of hydroxyl groups around the (C_1-O_{16}), (C_3-O_{17}), (C_4-O_{18}), ($C_{12}-O_{22}$), ($C_{13}-O_{23}$), ($C_{14}-O_{24}$), and ($C_{15}-O_{25}$) bonds, and the rotation around the ($C_{11}-C_{12}$), (C_7-O_{20}), and ($C_{10}-O_{20}$) bonds, according to the numbering presented in Figure 1. The potential energy surface was then explored from this starting point, along all the torsion angles of the backbone and the O−H bond orientations, without geometry constraint. For each cationized structure, the 10 oxygen atoms were considered as possible cationization sites: the starting geometry is that of the most stable neutral harpagide to which a H^+ or Na^+ cation is bound to an oxygen atom. Ten conformational searches were thus performed for both $HarH^+$ and $HarNa^+$. The Monte Carlo parameters were the following: a Metropolis criterion was used with a temperature of 400 K and a RMS gradient of 0.01 kcal/(Å mol). One to eight dihedral angles were simultaneously changed with torsion variations from ±30° to 180°. The optimized structures were considered to be duplicates if the difference in energy between two structures was less than 0.1 kcal/mol or if the varied torsion was within 5° or if the RMS error was within 0.25 Å. Each conformational search was stopped after 1000 successfully completed geometry optimizations.

DFT (Density Functional Theory) Calculations. The most stable structure of Har and the most stable conformer of each one of the 10 cationized forms of $HarH^+$ and of the 4 cationized forms of $HarNa^+$ (see below) were submitted to DFT calculations. Geometry optimizations and vibrational frequency calculations were carried out at the B3LYP/6-31G* level in order to check that optimized structures correspond to true minima on the potential energy surface. These calculations were also used to obtain zero point vibrational energies (ZPVE) and thermal corrections at 298 K (E_{therm}). Except as otherwise stated, the relative energies mentioned in the text and in the tables include the E_{therm} correction and are given in kcal/mol. We verified that thermal corrections do not change the energetic ordering of isomers. In order to calculate the proton affinity and the sodium binding affinity of harpagide, final energetics of the most stable conformer of Har, $HarH^+$, and $HarNa^+$ were obtained with B3LYP/6-311+G(2d,2p) wave functions at the optimized geometries. The Gaussian 03 program package[13] was used throughout. Optimized geometries are available upon request.

Results and Discussion

PA(Har) and SA(Har) are defined as the enthalpies of reactions 2 and 3, respectively:

$$HarH^+ \rightarrow Har + H^+ \quad (2)$$

$$HarNa^+ \rightarrow Har + Na^+ \quad (3)$$

$$PA(Har) = -\Delta_f H_{298}(HarH^+) + \Delta_f H_{298}(Har) + \Delta_f H_{298}(H^+)$$

$$SA(Har) = -\Delta_f H_{298}(HarNa^+) + \Delta_f H_{298}(Har) + \Delta_f H_{298}(Na^+)$$

The calculation of PA(Har) and SA(Har) requires the determination of the most stable conformation of Har, $HarH^+$, and $HarNa^+$. It led us to establish the preferential binding for H^+ and Na^+ sites in harpagide.

Geometry of the Neutral Form. Previous RMN studies[14,15] carried out on harpagide extracted from *H. procumbens* indicated that the configuration of asymmetric carbon atoms is that displayed in Figure 2, which shows the geometry of the most stable conformer according to our calculations. It is to be noted that the geometry optimization performed at the DFT level did not significantly change the geometric parameters of the most stable conformer obtained by AM1 optimization.

As displayed in Figure 2, the glucose is in the expected chair conformation. The $C_1-C_8-C_4-C_5$ and $C_3-C_4-C_8-C_7$ dihedral angle values are 101.5° and 153.0°, respectively. $C_8-C_7-O_{20}-C_{10}$ and $C_7-O_{20}-C_{10}-C_{15}$ dihedral angles were optimized at 97.2° and −72.3°, respectively. The hydrogen atom of the $O_{23}-H$ hydroxy group forms a hydrogen bond with O_{24} while the one of $O_{24}-H$ forms a hydrogen bond with O_{25}. The hydrogen atoms of $O_{17}-H$ and $O_{25}-H$ hydroxy groups are

TABLE 1: Relative Energies of the Most Stable Conformers Resulting from Protonation of Each Oxygen Atom of Harpagide with Both AM1 and B3LYP/6-31G* Calculation Levels[a]

	$HarH^+_{16}$	$HarH^+_{17}$	$HarH^+_{18}$	$HarH^+_{19}$	$HarH^+_{20}$	$HarH^+_{21}$	$HarH^+_{22}$	$HarH^+_{23}$	$HarH^+_{24}$	$HarH^+_{25}$
AM1	1.8	5.3	0.0	11.9	3.0	6.9	1.4	18.0	4.4	4.4
B3LYP/6-31G*	0.0	12.6	2.2	21.7	*b*	7.8	7.4	17.9	*c*	2.8

[a] Values are given in kcal/mol. [b] The proton on O_{20} shifted to O_{22} during geometry optimization of $HarH^+_{20}$ so that geometry optimizations of $HarH^+_{20}$ and $HarH^+_{22}$ led to the same structure. [c] In the same way, the proton on O_{24} shifted to O_{18} during geometry optimization of $HarH^+_{24}$.

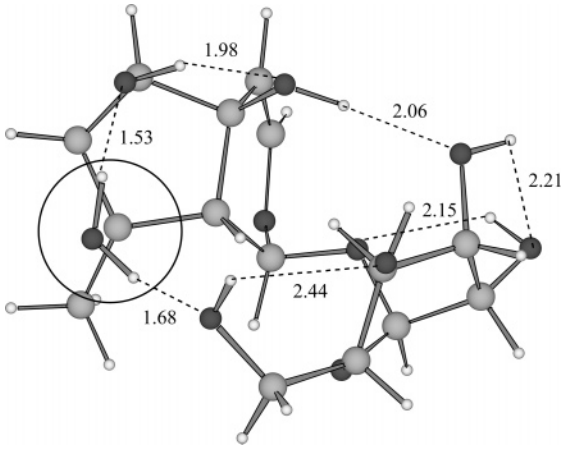

Figure 3. The most stable structure of protonated harpagide, optimized at the B3LYP/6-31G* level: $HarH^+_{16}$. The protonation site is circled. Hydrogen bond lengths (in dotted lines) are in Å.

involved in hydrogen bonds with O_{18} (hydrogen bond lengths are given in Figure 2).

Geometry of the Protonated Form. The total and relative energies of the most stable conformers resulting from the protonation of each oxygen atom are given in Table 1, at the semiempirical AM1 level and at the B3LYP/6-31G* level. They are named $HarH^+_x$, where *x* is the number (see Figure 1) of the protonated oxygen atom. At the AM1 level, no proton was transferred during geometry optimization. At the B3LYP/6-31G* level, the geometry optimization of $HarH^+_{24}$ shifted the proton from O_{24} to O_{18} and that of $HarH^+_{20}$ shifted the proton from O_{20} to O_{22}, leading to eight distinct structures. Table 1 shows that the protonation on O_{18} provided the most stable structure according to AM1 calculations while DFT calculations gave $HarH^+_{16}$ as the most stable one. The B3LYP results show that the most favorable protonation sites are O_{16}, O_{18}, and O_{25}. With the AM1 method, these sites are also the most likely to be be protonated (although in a different order) but O_{20}, O_{22}, and O_{24} are found to be also favored. This latter result shows the insufficient reliability of semiempirical calculations to compare the relative energies of isomers of such molecules. However, the AM1 method can be used to compare efficiently the conformers for a given cationization site as described in the Method Validation section.

$HarH^+_{16}$ has been retained as the most stable structure of protonated harpagide. Its geometry optimized at the B3LYP/6-31G* level is displayed on Figure 3. It shows that the most stable geometry of protonated harpagide is very different from that of the neutral molecule. Both hydrogen atoms on O_{16} are involved in hydrogen bonds: the first one with O_{17}, the second one with O_{22}. The latter and the hydrogen bond between the hydrogen of $O_{18}-H$ with O_{24} lead the molecule to fold up. The $C_1-C_8-C_4-C_5$ and $C_3-C_4-C_8-C_7$ dihedral angle values are 105.5° and −143.8°, respectively. $C_8-C_7-O_{20}-C_{10}$ and $C_7-O_{20}-C_{10}-C_{15}$ dihedral angles were optimized at 107.7° and −171.0°, respectively. The structure is stabilized by seven hydrogen bonds, including the three ones mentioned above. The hydrogen atom of the $O_{17}-H$ hydroxy group forms a hydrogen bond with O_{18}, the hydrogen of $O_{22}-H$ with O_{23}, the hydrogen of $O_{24}-H$ with O_{25}, and that of $O_{25}-H$ with O_{20} (hydrogen bond lengths are given on Figure 3).

TABLE 2: Relative Energies at AM1, B3LYP/6-31G*, and B3LYP/6-311+G(2d,2p)//B3LYP/6-31G* Calculation Levels for the Most Stable Conformers of Na^+-Cationized Harpagide[a]

Na^+ coordination	$HarNa^+_I$ O_{18}, O_{20}, O_{24}, O_{25}	$HarNa^+_{II}$ O_{18}, O_{20}, O_{21}, O_{22}	$HarNa^+_{III}$ O_{16}, O_{17}, O_{22}	$HarNa^+_{IV}$ O_{16}, O_{20}, O_{21}, O_{22}
AM1	3.3	0.0	5.3	3.2
B3LYP/6-31G*	0.0	1.7	3.4	12.6
B3LYP/6-311+G(2d,2p)//B3LYP/6-31G*	0.0	3.4	5.9	10.0

[a] Values are given in kcal/mol.

Geometry of the Na^+-Cationized Form. Geometry optimizations of harpagide$-Na^+$ complexes were carried out in the same way as for $HarH^+$ ions, i.e. performing Monte Carlo conformational searches from 10 starting points, each one corresponding to cationization on a different oxygen atom. The starting points are named $HarNa^+_x$, where *x* is the number (see Figure 1) of the oxygen atom which is cationized. It is to be noted that conformational searches performed on $HarNa^+_{18}$ to $HarNa^+_{24}$ provided minima very close to each other in energies and structures. DFT optimization of these minima led to the same structure, named $HarNa^+_{II}$ (see below), in which the Na^+ ion is chelated between four oxygen atoms: O_{18}, O_{20}, O_{21}, and O_{22}. $HarNa^+_I$ results from the conformational search on $HarNa^+_{25}$; in this structure, the sodium ion is bonded to O_{18}, O_{20}, O_{24}, and O_{25}. $HarNa^+_{III}$, in which Na^+ is bonded to O_{16}, O_{17}, and O_{22}, corresponds to the most stable structure obtained from the conformational search from $HarNa^+_{17}$. $HarNa^+_{IV}$ results from the conformational search of $HarNa^+_{16}$: Na^+ is complexed to O_{16}, O_{20}, O_{21}, and O_{22}. The geometries optimized are consistent with previous work by Cerda and Wesdemiotis, who studied the multidentate coordination of sodium ion by the oxygen sites of sachacharides and showed that Na^+ is usually attached to three or four oxygen atoms.[16] In the same way, an ab initio study by Botek et al. devoted to cationization of glucopyranose showed that Na^+ is coordinated to four oxygen atoms.[17] Although multidentate binding was expected for harpagide as well, we chose to start with all 10 possible monodentate structures in order to avoid as much as possible a priori biases, and to explore the potential energy surface in a comprehensive manner. The fact that the 10 starting structures converged to only four distinct minima is an indication that the structures obtained are the most stable. The relative energies and the oxygen atoms involved in Na^+ complexation in the four resulting minima, namely, $HarNa^+_I$ to $HarNa^+_{IV}$, are given in Table 2. The energy ordering of the four complexes is not the same for AM1 and DFT calculations, as already noted for $HarH^+$ structures. Given the small energy differences between the four minima, further single point calculations were performed at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G* level. Semiquantitative agreement is obtained between both DFT levels. $HarNa^+_I$ corresponds to the most stable isomer; it has been retained for the determination of sodium affinity of harpagide.

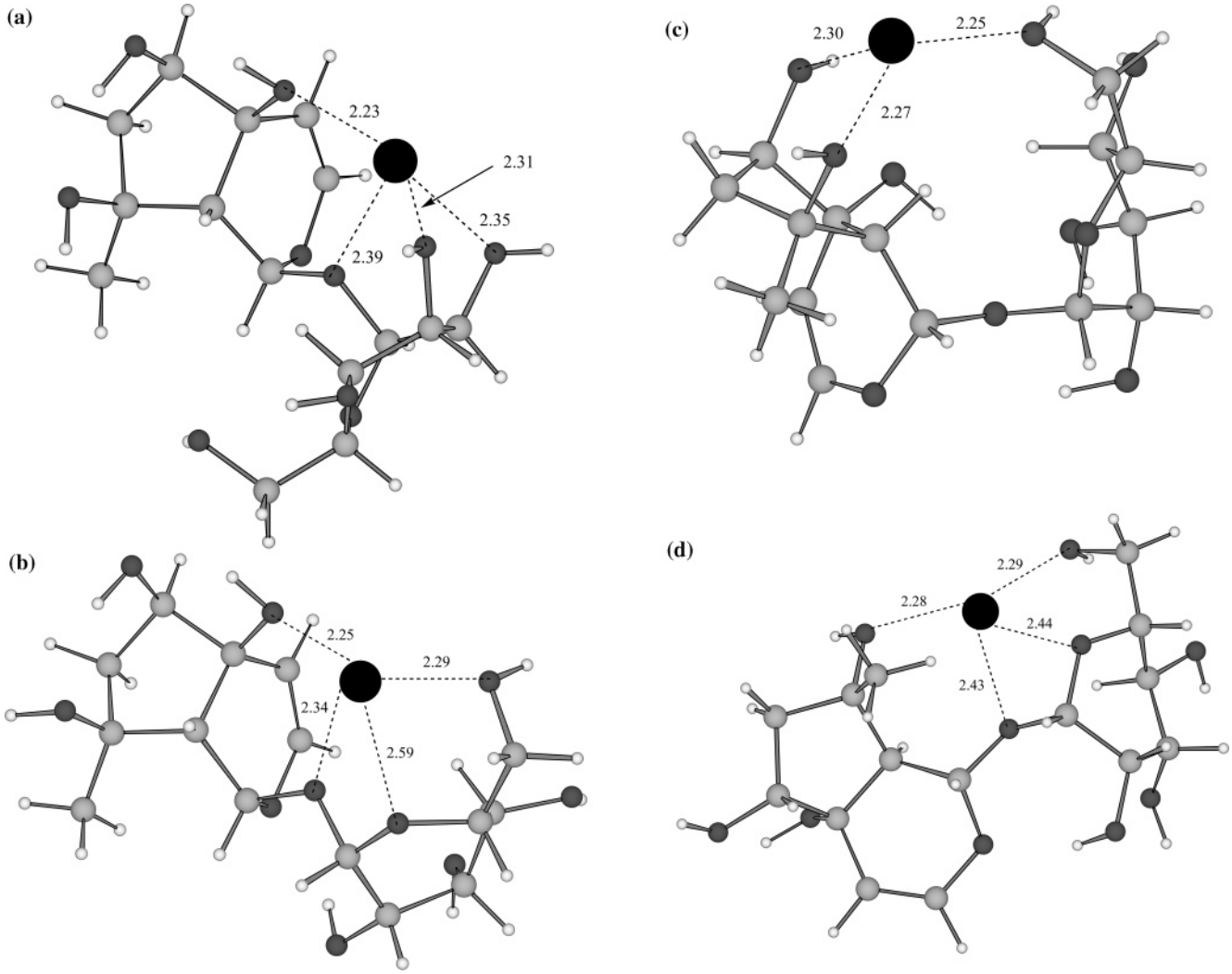

Figure 4. (a) Geometry of $HarNa^+_I$ optimized at the B3LYP/6-31G* level; distances are in Å. (b) Geometry of $HarNa^+_{II}$ optimized at the B3LYP/6-31G* level; distances are in Å. (c) Geometry of $HarNa^+_{III}$ optimized at the B3LYP/6-31G* level; distances are in Å. (d) Geometry of $HarNa^+_{IV}$ optimized at the B3LYP/6-31G* level; distances are in Å.

Figure 4a−d displays the most stable structures, $HarNa^+_I$ to $HarNa^+_{IV}$, optimized at the B3LYP/6-31G* level. Based on the computed geometries for Na^+−alcohol and Na^+−ether species,[18,19] we consider that Na^+ is bound to an oxygen atom if the O−Na^+ distance is smaller than 2.7 Å. In $HarNa^+_I$, Na^+ is bound to four oxygen atoms: O_{18}, O_{20}, O_{24}, and O_{25}. The hydrogen atom of the O_{18}−H hydroxy group forms a hydrogen bond (1.91 Å) with O_{17}, that of O_{17}−H with O_{16} (1.94 Å). An electrostatic interaction is also involved between the hydrogen atom of the O_{24}−H hydroxy group and O_{23} (distance H- - -O_{23} = 2.63 Å). In $HarNa^+_{II}$, Na^+ is bonded to O_{18}, O_{20}, O_{21}, and O_{22}. As in $HarNa^+_I$, the hydrogen atom of the O_{18}−H hydroxy group forms a hydrogen bond (1.90 Å) with O_{17}, that of O_{17}−H with O_{16} (1.96 Å). $HarNa^+_{III}$ is the only low-energy isomer found where Na^+ is complexed to only three oxygen atoms: O_{16}, O_{17}, and O_{22}. Two hydrogen bonds are involved in $HarNa^+_{III}$: one between the hydrogen atom of the O_{17}−H hydroxy group and O_{18} (2.08 Å) and one between the hydrogen atom of the O_{22}−H hydroxy group and O_{23} (2.05 Å). In $HarNa^+_{IV}$, Na^+ is complexed to O_{16}, O_{20}, O_{21}, and O_{22}. The hydrogen atom of the O_{18}−H hydroxy group forms a hydrogen bond (2.12 Å) with O_{17}, that of O_{25}−H with O_{19} (1.88 Å).

Method Validation

The aim of the Monte Carlo conformational searches is to find the most stable structure of $HarH^+$ and that of $HarNa^+$, for the determination of PA(Har) and SA(Har), respectively. Therefore, it is to be remarked that the second or third "best" geometry for a given $HarH^+$ or $HarNa^+$ isomer might have an energy lower than the most stable geometry of an other isomer. It could lead to omission of the true minimum if DFT calculations modify the stability order for the AM1 minima of a given isomer. In order to check that the energy orders of conformers are the same according to both levels of calculation, the three geometries of lowest energies found for each of the isomers of $HarH^+_{16}$, $HarH^+_{25}$, and $HarNa^+_{18}$ by AM1 conformational searches were also computed at the DFT level. Relative energies of protonated and cationized conformers are compared in Table 3.

TABLE 3: Relatives Energies, in kcal/mol, of the Three Most Stable Conformers of $HarH^+_{16}$, $HarH^+_{25}$, and $HarNa^+_{18}$ at Both AM1 and B3LYP/6-31G* Levels

	$HarH^+_{16}$			$HarH^+_{25}$			$HarNa^+_{18}$		
	min 1	min 2	min 3	min 1	min 2	min 3	min 1	min 2	min 3
AM1	0.0	0.8	1.2	0.0	3.7	5.4	0.0	0.2	1.0
B3LYP/6-31G*	0.0	1.3	4.3	0.0	0.6	3.4	0.0	0.8	2.6

According to values in Table 3, semiempirical and DFT results are in good agreement concerning the stability order of

TABLE 4: Total Energies, E (B3LYP/6-311+G(2d,2p)// B3LYP/6-31G*), and Thermal Corrections at 298 K, E_{therm} (B3LYP/6-31G*), of the Most Stable Structure of Each Species

species	E (hartree)	E_{therm} (kcal/mol)
H^+	0	0.9
Na^+	−162.087523	0.9
Har	−1338.670194	270.5
$HarH^+$	−1339.037162	278.7
$HarNa^+$	−1500.863430	272.3

conformers. It is therefore very unlikely that the overall minimum has been missed with the computational procedure used.

Determination of Proton and Cation Sodium Affinities

The total energies determined at the B3LYP/6-311+G(2d,-2p)//B3LYP/6-31G* level for H^+, Na^+, Har, $HarH^+$, and $HarNa^+$ are given in Table 4.

Calculation of the proton affinity of Har is done as follows:

$$PA(Har) = -[E(HarH^+) + E_{therm}(HarH^+) + RT] + [E(Har) + E_{therm}(Har) + RT] + [E(H^+) + E_{therm}(H^+) + RT]$$

Using the results displayed in Table 4, the proton affinity of harpagide is calculated as PA(Har) = 223.5 kcal/mol. In the same manner, the sodium ion affinity of harpagide is calculated as SA(Har) = 66.0 kcal/mol.

The computed sodium ion affinity of harpagide is significantly smaller than the sum of the affinities of methanol molecules in $Na^+(CH_3OH)_4$ (66.0 vs about 80.0 kcal/mol),[20] even though the number of oxygens is the same and the functional groups are similar. This is due to the fact that harpagide has to fold to provide several binding sites simultaneously, which has an intramolecular energy cost. In addition, this does not allow for optimum binding of each individual site. Previous calculations on molecules with analogous binding oxygens have already underlined this feature. For instance, a 1,2-diol or a 1,3-diol cannot bind as strongly as two individual alcohol molecules,[18,19] and glycerol has a binding interaction with Na^+ that is much smaller than those of 3 methanol molecules (44.0 vs about 65.0 kcal/mol).[19,20] The SA computed for harpagide is not much different from the value determined for monosaccharides by Cerda and Wesdemiotis.[16]

The calculation of the $\Delta_r H_{298}$ of reaction 1, $\Delta_r H_{298}(1)$ = SA-(MeOH) + PA(Har) − PA(MeOH) − SA(Har), requires PA-(MeOH) and SA(MeOH) values. The literature values for PA(MeOH) and SA(MeOH) are 180.3 kcal/mol[21] and 24.0 kcal/mol, respectively;[18] they were retained for the calculation of $\Delta_r H_{298}(1)$. At the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G* level, we determined PA(MeOH) = 179.2 kcal/mol and SA-(MeOH) = 25.2 kcal/mol, in good agreement with literature data. $\Delta_r H_{298}(1) = 24.0 + 223.5 - 180.3 - 66.0 = 1.2$ kcal/mol. $\Delta_r S_{298}(1)$ was calculated as $\Delta_r S_{298}(1) = S_{298}(MeOH_2^+) + S_{298}(HarNa^+) - S_{298}(MeOHNa^+) - S_{298}(HarH^+)$. Our calculations gave $\Delta_r S_{298}(1) = 58.36 + 162.19 - 70.04 - 152.71 = -2.2$ cal·mol^{-1}·K^{-1}. $\Delta_r G_{298}(1)$ can be calculated as $\Delta_r G_{298}(1) = \Delta_r H(1) - T\Delta_r S_{298}(1) = 1.2 - [298.15(-2.2)/1000] = 1.9$ kcal/mol. Such a value is in the limit of the accuracy of the method and does not allow us to conclude definitely whether reaction 1 is endo- or exothermic. Given this very small value, reaction 1 is expected to proceed easily in the final stages of the ion desolvation process. Thus it is conceivable that harpagide is under its protonated form in solution, and that a cation transfer reaction occurs in the gas phase, leading to the observation of $HarNa^+$ as the predominant pseudo-molecular ion.

Microsolvation: Effect of a Methanol Molecule Complexation to Each Reagent and Product of Reaction 1

It is to be noted that reaction 1 does not take into account any solvation effect. Let us consider reaction 4 corresponding to reaction 1 to which a methanol molecule has been complexed to each reagent and product.

$$(MeOH)_2Na^+ + [Har, MeOH, H^+] \rightarrow (MeOH)_2H^+ + [Har, MeOH, Na^+] \quad (4)$$

Calculation of $\Delta_r H_{298}(4)$ requires the knowledge of the $\Delta_r H_{298}$ for reactions (5) to (8):

$$(MeOH)H^+ + MeOH \rightarrow (MeOH)_2H^+ \quad (5)$$

$$(MeOH)Na^+ + MeOH \rightarrow (MeOH)_2Na^+ \quad (6)$$

$$HarH^+ + MeOH \rightarrow [Har, MeOH, H^+] \quad (7)$$

$$HarNa^+ + MeOH \rightarrow [Har, MeOH, Na^+] \quad (8)$$

Literature data provide values of −32.6 kcal/mol[22] and −20.5 kcal/mol[23] for $\Delta_r H_{298}(5)$ and $\Delta_r H_{298}(6)$, respectively. Geometries of the complexes [Har, MeOH, H^+] and [Har, MeOH, Na^+] were optimized at the B3LYP/6-31G* level from the most stable structures of $HarH^+$ and $HarNa^+$ to which a methanol molecule was added. The resulting structures are displayed in Figure 5a,b. [Har, MeOH, H^+] is a proton-bound dimer of Har and MeOH, with a shorter O···H^+ bond on the Har side (1.10 vs 1.35 Å), consistent with the larger proton affinity of Har. The hydroxyl group of methanol also makes a strong hydrogen bond with O_{22} (1.67 Å). Thus introducing the methanol molecule into the structure of $HarH^+_{16}$ makes a hydrogen bond network between O_{16} and O_{22}, leading to a significant stabilization. The structure of $HarNa^+_I$ is such that an additional methanol molecule can easily bind to Na^+. Still, the complexation of methanol to Na^+-cationized harpagide leads to an increase of the distances between Na^+ and the four oxygen atoms to which Na^+ is bonded, of 0.06 to 0.08 Å (see Figures 4a and 5b). This accounts for the relief of steric repulsions between ligands in the more crowded sodium environment. $\Delta_r H_{298}(7)$ and $\Delta_r H_{298}(8)$ were estimated at the B3LYP/6-311+G(2d,2p) level to be −19.4 and −9.7 kcal/mol, respectively. The binding enthalpy of the additional methanol molecule on $HarNa^+$ is comparable to that of the fifth and sixth molecules in $Na^+(CH_3OH)_n$, which have been computed to be 6.5 and 12.3 kcal·mol^{-1}, respectively.[20]

$\Delta_r H_{298}(4)$ can be calculated as $\Delta_r H_{298}(4) = 1.2 - 32.6 + 20.5 + 19.4 - 9.7 = -1.2$ kcal/mol. $\Delta_r S_{298}(4)$ can be calculated as $\Delta_r S_{298}(4) = \Delta_r S_{298}(1) + \Delta_r S_{298}(5) - \Delta_r S_{298}(6) - \Delta_r S_{298}(7) + \Delta_r S_{298}(8) = -2.2 - 29.0 + 21.7 + 33.1 - 21.8 = 1.8$ cal·mol^{-1}·K^{-1}. $\Delta_r S_{298}(1)$, $\Delta_r S_{298}(7)$, and $\Delta_r S_{298}(8)$ are issued from our calculations while $\Delta_r S_{298}(5)$ and $\Delta_r S_{298}(6)$ values were taken from the literature.[22,24] $\Delta_r G_{298}(4) = \Delta_r H_{298}(4) - T\Delta_r S_{298}(4) = -1.2 - (298.15 \times 1.8/1000) = -1.7$ kcal/mol. Complexing a methanol molecule to the reagents and products of reaction 1 makes it become slightly exothermic but does not change the reaction enthalpy significantly. It is likely that further solvation would not change the conclusion that reaction 1 is relatively easy at room temperature.

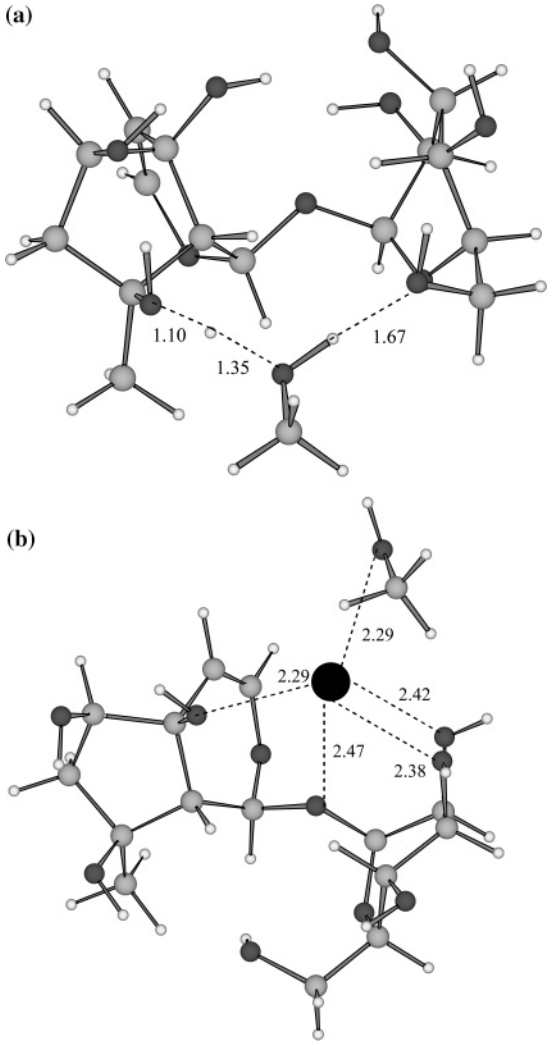

Figure 5. (a) Geometry of $HarH^{+}_{16}$ complexed to a methanol molecule optimized at the B3LYP/6-31G* level; distances are in Å. (b) Geometry of $HarNa^{+}_{I}$ complexed to a methanol molecule optimized at the B3LYP/6-31G* level; distances are in Å.

It is to be remarked that a model using only two molecules of methanol is certainly insufficient given the enormous capacity of harpagide to form hydrogen bonds with the solvent, even if it is likely that some of the interactions between harpagide and methanol molecules would cancel out comparing the protonated and the cationized forms. Given its insufficiency, the model just allows one to conclude that the proton/sodium exchange reaction is quasi thermoneutral at the end of the desolvation step, as in the gas phase; it does not allow one to conclude about the feasibility of proton/sodium ion exchange in bulk solvent.

Conclusion

This study allowed the establishment of the most stable conformations of harpagide and its protonated and sodium-cationized forms. Proton and sodium cation affinities of harpagide were estimated to be 223.5 and 66.0 kcal/mol, respectively. The cation transfer reaction of sodium and proton between harpagide and methanol, yielding protonated methanol and sodiated harpagide, is slightly endothermic by 1.9 kcal/mol. Complexing a methanol molecule to each reagent and product (i.e. considering $(MeOH)_2Na^+$ and a complex [Har, MeOH, H^+] as reagents and $(MeOH)_2H^+$ and a complex [Har, MeOH, Na^+] as products) makes the reaction become slightly exothermic by 1.7 kcal/mol. These small values are in the limit of accuracy of the method; they suggest that cationization of harpagide is easily feasible either in solution or within electrospray droplets as long as Na^+ is available in the solution. Yet, this result does not allow one to discard the possibility that harpagide is in a sodium salt form ("preformed" $HarNa^+$ ions) in the commercial product and in biological samples.

References and Notes

(1) Andersen, M. L.; Santos, E. H. R.; Seabra, M. d. L. V.; Da Silva, A. A. B.; Tufik, S. *J. Ethnopharmacol.* **2004**, *91* (2−3), 325−330.

(2) Chantre, P.; Cappelaere, A.; Leblan, D.; Guedon, D.; Vandermander, J.; Fournie, B. *Phytomedicine* **2000**, *7*, 177−183.

(3) Gagnier, J. J.; Chrubasik, S.; Manheimer, E. *BMC Complementary Altern. Med.* **2004**, *4* (1), 13.

(4) Mahomed, I. M.; Ojewole, J. A. *Phytother. Res.* **2004**, *18*, 982−989.

(5) Baranska, M.; Schultz, H.; Siuda, R.; Strehle, M. A.; Rosch, P.; Popp, J.; Joubert, E.; Manley, M. *Biopolymers* **2005**, *77*, 1−8.

(6) Manuscript submitted for publication.

(7) (a) Harvey, D. J. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 900−915. (b) Harvey, D. J. In *Encyclopedia of Mass Spectrometry*; Elsevier: New York, 2005; Vol. 4, pp 584−593.

(8) Orlando, R.; Bush, C. A.; Fenselau, C. *Biomed. Environ. Mass Spectrom.* **1990**, *19*, 747−754.

(9) Amad, M. H.; Cech, N. B.; Jackson, G. S.; Enke, C. G. *J. Mass Spectrom.* **2000**, *35*, 784−789.

(10) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. P. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.

(11) Brothers, E.; Merz, K. M., Jr. *J. Phys. Chem. B* **2002**, *106*, 2779−2785.

(12) HyperChem (TM) Professional 7.51, Hypercube, Inc., 1115 NW 4th Street, Gainesville, FL 32601.

(13) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision B.05; Gaussian, Inc.: Pittsburgh, PA, 2003.

(14) Kikuchi, T.; Matsuda, S.; Kubo, Y.; Namba, T. *Chem. Pharm. Bull.* **1983**, *31*, *1* (7), 2296−2301.

(15) Seger, C.; Godejohann, M.; Tseng, L. H.; Spraul, M.; Girtler, A.; Sturm, S.; Stuppner, H. *Anal. Chem.* **2005**, *77*, 878−885.

(16) Cerda, B. A.; Wesdemiotis, C. *Int. J. Mass Spectrom.* **1999**, *189*, 189−204.

(17) Botek, E.; Debrun, J. L.; Hakim, B.; Morin-Allory, L. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 273−276.

(18) (a) McMahon, T. B.; Ohanessian, G. *Chem. Eur. J.* **2000**, *6* (16), 2931−2941. (b) Hoyau, S.; Norrman, K.; McMahon, T. B.; Ohanessian, G. *J. Am. Chem. Soc.* **1999**, *121*, 8864−8875. (c) Armentrout, P. B.; Rodgers, M. T. *J. Phys. Chem. A* **2000**, *104*, 2238−2247. (d) Bloomfield, J.; Davies, E.; Gatt, P.; Petrie, S. *J. Phys. Chem. A* **2006**, *110*, 1134−1144.

(19) Albirami, S.; Ma, N. L.; Groh, N. K. *Chem. Phys. Lett.* **2002**, *359*, 500−506.

(20) Garcia-Muruais, A.; Cabaleiro-Lago, E. M.; Hermida-Ramon, J. M.; Rios, M. A. *Chem. Phys.* **2000**, *254*, 109−123.

(21) Hunter, E. P.; Lias, S. G. *J. Phys. Chem. Ref. Data* **1998**, *27* (3), 413−656.

(22) Meot-Ner, M. *J. Am. Chem. Soc.* **1992**, *114*, 3312−3322.

(23) Amicangelo, J. C.; Armentrout, P. B. *Int. J. Mass Spectrom.* **2001**, *212*, 301−325.

(24) Guo, B. C.; Conklin, B. J.; Castleman, A. W. *J. Am. Chem. Soc.* **1989**, *111*, 6506.