Conformational and Cs^+ complexation properties of norbadione-A: a molecular modeling study†‡

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We report a quantum mechanical (QM) and classical molecular dynamics (MD) study of the conformational and complexation properties of norbadione-A (NBA), a key pigment involved in the Cs^+ complexation by mushrooms. The *Z* versus *E* isomers of its pulvinic moieties are compared in their neutral (Pulv^0), mono- (Pulv^{-1}) and di-deprotonated (Pulv^{-2}) states, and the ^1H chemical shifts are calculated *ab initio*. Pulv^{-1} is found to be stabilized in the *E* form by an internal $\text{COOH}\cdots\text{O}(\text{enolate})$ hydrogen-bond. No energy minimum is found for the corresponding $\text{COO}^-\cdots\text{HO}(\text{enol})$ state, indicating that the conjugated enol function of Pulv^0 is more acidic than the COOH function. Further deprotonation leads to the *Z* and *E* forms of Pulv^{-2} that are close in energy and both account for a marked downfield shift δ of *ortho*- H_8 protons. A similar shift is found upon deprotonation of the enol function of an ester analogue of Pulv^0 . Therefore, contrary to previous assumptions (ref. 7: P. Kuad, *et al.*, *J. Am. Chem. Soc.*, 2005, **127**, 1323), the large shift of $\delta(\text{H}_8)$ around pH 9.5 upon deprotonation of NBA or of pulvinic acid cannot be taken as an indicator of an *E*-to-*Z* conformational switch, but merely reflects the pH-induced conformational change of the carboxylate group adjacent to the (H_8)-ring. The QM and MD studies on NBA^{2-} and NBA^{4-} support the view that both species prefer the *E/E* form with two intramolecular $\text{COOH}\cdots\text{O}(\text{enolate})$ hydrogen-bonds in the gas phase and in solution. Finally, we simulated mono- and di-nuclear complexes of Cs^+ with NBA^{2-} and NBA^{4-} by MD, showing that only the NBA^{4-} state populated at high pH values can bind two Cs^+ cations, with both *E* and *Z* conformations of the pulvinic arms.

Introduction

Norbadione-A (NBA; see Fig. 1), a natural pigment extracted from the brown pileus of the bay boletus,^{1,2} is presumed to complex with caesium cations selectively and to be, therefore, the main one responsible for the high level of ^{137}Cs radioactivity detected in edible mushrooms after the reactor accident at Chernobyl. Moreover, NBA, remarkably, displays pronounced atypical anti-radiation properties by protecting DNA-related targets from irradiation.^{3,4} Due to both their caesium-complexation ability and radiation-protecting properties, NBA and related compounds may be potential candidates for caesium decorporation of contaminated persons. In spite of the fundamental, and possibly technological, importance of these compounds, little is known about their structural and complexation properties. The NBA molecule carries

seven functional groups that render it and its complexes somewhat water-soluble, and complexation studies of Cs^+

Fig. 1 Norbadione-A in its neutral form, and deprotonated $\text{NBA}_{\text{enol}}^{2-}$, $\text{NBA}_{\text{COOH}}^{2-}$ and NBA^{4-} forms.

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† This work is dedicated to the memory of Dr Charles Mioskowski who too early passed away. He was a great scientist, recognized worldwide for his outstanding contributions to bioorganic chemistry.

‡ Electronic supplementary information (ESI) available: Total energies and chemical shifts of the various complexes (Tables S1–S3); a CP-MD simulation (Fig. S1); optimized structures (Fig. S2), chemical shifts (Fig. S3 and S5), relative energies (Fig. S4) and final snapshots of the various isomers (Fig. S5–S9). See DOI: 10.1039/b712836c

cations by NBA in methanol–water mixtures have recently been published by two teams.^{5,6} Their conclusions, which mainly rest on electrospray mass spectroscopic data, diverge in the nature of the complexed species that occur, as well as in their stability constants. Both studies, however, agree that the complexation mechanism is uncommon. A detailed investigation of the acid–base behaviour at the intramolecular level has also been reported,⁷ pointing to the importance of a pH-dependent *Z*-to-*E* isomer switch that occurs in both pulvinic moieties of NBA, yielding four stereomeric forms (*E/E*, *E/Z*, *Z/E* and *Z/Z*), the complexation ability of which for metallic cations may differ largely in solution. In addition, the results showed that the acid–base properties of NBA are strongly dependent on its ionic environment, even though it is made up with tetraethylammonium perchlorate (Et₄NClO₄), whose large anion and cation are usually presumed to have a negligible influence on the protonation constants.

In this paper we report molecular modeling studies performed along two main directions. First, we investigate the conformational preferences of a key fragment of NBA, *i.e.* a pulvinic acid, the structure of which is fully optimized in the gas phase using quantum mechanics calculations (QM), considering the neutral, mono- and di-deprotonated forms, hereafter denoted Pulv2⁰, Pulv2^{−1} and Pulv2^{−2}, respectively (keeping the same index “2” as in ref. 7; see Fig. 2). An ester analogue Pulv3 with an ionizable enol function (noted “3” in ref. 7) will also be considered (Fig. 2). This QM study allows us to address the question of co-planarity of the different building units (*i.e.* the five-membered ring lactone, the carboxylate and the two anisole groups) and to compare the *E* versus *Z* isomers generated by the C=C bond. These calculations also shed light on the localization of the acidic proton in the Pulv2^{−1} species (*i.e.* on the carboxylic acid versus enol groups). The ¹H NMR chemical shifts are also calculated *ab initio* in the different structures of the pulvinic derivatives and norbadione, with the main aim being to assess whether the large shift of H₈ protons of the anisole ring adjacent to the carboxylate functions (see Fig. 2) can be taken as indicator of a pH-induced *E* ↔ *Z* isomerization. Similar NMR calculations are performed on different *Z/E* isomers of norbadione itself in its NBA^{2−} and NBA^{4−} deprotonated states (Fig. 1). In a second part of the paper we use classical molecular dynamics (MD) simulations to model different states of the norbadione molecule, as well as possible structures of their Cs⁺ complexes in the gas phase and in methanol solution.

Methods

Ab initio calculations

Quantum mechanics (QM) calculations have been performed on different protonation states of the norbadione molecule (NBA^{2−} and NBA^{4−}; see Fig. 1) and on the Pulv2 and Pulv3 pulvinic acid derivatives with the Gaussian 03 software⁸ at the HF/6-31G(d,p)//HF/6-31G(d) level of theory. ¹H NMR chemical shifts δ have been calculated at the HF/6-31G(d,p) level on all compounds. Furthermore, tests using the DFT instead of the HF method (B3LYP/6-311G⁺⁺(d,p) calculations) have been performed to obtain δ on the small systems, namely the Pulv2 and Pulv3 molecules in their neutral and deprotonated states.

Classical molecular dynamics simulations

The MD simulations were performed with the AMBER 5.0 software⁹ where the potential energy is described by a sum of bond-, angle- and dihedral-deformation energies, and pair-wise additive 1-6-12 (electrostatic + van der Waals) interactions between non-bonded atoms.

$$U = \sum_{\text{bonds}} K_r (r - r_{\text{eq}})^2 + \sum_{\text{angles}} K_\theta (\theta - \theta_{\text{eq}})^2 + \sum_{\text{dihedrals}} V_n (1 + \cos n\phi) + \sum_{i < j} \left(\frac{q_{ij}}{R_{ij}} - 2\epsilon_{ij} \left(\frac{R_{ij}}{R_{ij}^6} \right) + \epsilon_{ij} \left(\frac{R_{ij}}{R_{ij}^{12}} \right) \right)$$

The Cs⁺ parameters were from Åqvist.¹⁰ Methanol was represented with the OPLS model.¹¹ All O–H and C–H bonds were constrained with SHAKE, using a time step of 2 fs. The atomic charges used for the different NBA^{2−} and NBA^{4−} forms of the norbadione were derived from *ab initio* B3LYP/6-31G* electrostatic potentials. The V_n torsional potentials around C–C(anisole) dihedrals were set to zero to allow for “free rotation” of these dihedrals. The starting structures of the *Z* or *E* isomers were model-built with co-planar lactone and carboxylate groups.

The MD simulations were performed in the NVT ensemble where the number N of particles, the volume V and the temperature T are constant. The systems were represented with 3D periodic boundary conditions and the non-bonded interactions were calculated using a 14 Å cutoff, with a reaction field correction at the boundaries.¹² All systems were first minimized for 1000 steps, and then simulated for 1 ns in

Fig. 2 Pulvinic species in their neutral forms Pulv2⁰ and Pulv3⁰. The different deprotonated forms Pulv2^{−1} and Pulv2^{−2} are shown in Fig. 3.

the gas phase at 300 K. For the free norbadione ligand, the simulation at 300 K started after 1 ns of sampling at 900 K. The final structures at 300 K were immersed in a box of methanol solvent. After 1000 steps of energy minimization and 100 ps of dynamics at 100 K, we simulated the free ligand and its Cs^+ complexes for 1 to 2 ns.

Car–Parrinello molecular dynamics (CP-MD) simulations

CP-MD simulations were performed with the CPMD program,¹³ using the gradient-corrected BLYP functional,^{14,15} and norm-conserving Troullier–Martins pseudopotentials.^{16,17} Kohn–Sham orbitals were expanded in plane waves up to a kinetic energy cutoff of 80 Ry. The simulation was performed in the NVT ensemble using a single Nosé–Hoover thermostat set to 300 K, a fictitious electronic mass of 600 a.u., and a time step of 0.121 fs.

Results and discussion

(1) QM studies of the pulvinic acid (Pulv2) and its methylated ester analogue (Pulv3)

In this section, we report the main structural and energy features of the Pulv2 and Pulv3 molecules in their neutral states (Pulv2^0 and Pulv3^0) and deprotonated states (Pulv2^{-1} and Pulv2^{-2} , Pulv3^{-1} ; see Fig. 2). For each state, we compare

Fig. 3 HF/6-31G(d,p)//HF/6-31G(d) energy differences (in kcal mol⁻¹) between different forms of the neutral and deprotonated Pulv2 molecule ($R = -\text{C}_6\text{H}_4-\text{OCH}_3$).

the Z to the E isomers and, in the case of the Pulv2^{-1} species, we consider structures where the acidic proton is initially either on the enol ($\text{Pulv2}_{\text{enol}}^{-1}$) or the carboxylic ($\text{Pulv2}_{\text{COOH}}^{-1}$) group. The main energy results are summarized in Fig. 3, Table 1 and Table S1,† and optimized structures are shown in Fig. 4. Note that both HF/6-31G(d) and HF/6-31G(d,p) basis sets give similar energy differences ΔE , as does the DFT(B3LYP)/6-311G⁺⁺(d,p)//HF/6-31G(d,p) method (Table S1)† and, in the following, we only present the HF/6-31G(d,p)//HF/6-31G(d) results, for consistency with the norbadione results. They show that for the neutral Pulv2^0 molecule, the E form is preferred over the Z one (by 7.3 kcal mol⁻¹); the first deprotonation of Pulv2^0 leads to the $\text{Pulv2}_{\text{COOH}}^{-1}$ E form, while for the next deprotonated state (Pulv2^{-2}), the conformational preference is much less clear cut, the Z form being only 1.3 kcal mol⁻¹ lower in energy than the E form. There is thus no clear evidence for an E-to-Z conformational switch upon deprotonation of Pulv2^{-1} in the gas phase.

Regarding the different Pulv2^{-1} species, one sees that the total energies follow the order $E_{\text{COOH}} < Z_{\text{COOH}} < Z_{\text{enol}}$ ($\Delta E = 0.0, 6.7$ and 30.7 kcal mol⁻¹, respectively). The calculated rotation barrier exchanging the E to the Z isomers of the $\text{Pulv2}_{\text{COOH}}^{-1}$ amounts to 33 kcal mol⁻¹, whereas the Z-to-E barrier is lower (22 kcal mol⁻¹) due to the higher stability of the E isomer.¹⁸ In the case of the Pulv2^{-2} species the Z and E isomers, close in energy ($\Delta E = 1.3$ kcal mol⁻¹), are separated by a barrier of 33 kcal mol⁻¹.

Let us discuss in more detail the $\text{Pulv2}_{\text{COOH}}^{-1}$ structure which is stabilized in the E form *via* a strong internal hydrogen-bond between the acidic (CO)O–H proton and the adjacent enolate, forming a 7-membered ring with co-planar atoms. The resulting $\text{H}_{\text{COOH}} \cdots \text{O}_{\text{enolate}}$ distance is unusually short (1.530 Å). Surprisingly, the proton prefers the carboxylic acid over the enol group, and this feature has been confirmed by several computational tests. (i) First, no energy minimum could be located for the $\text{Pulv2}_{\text{enol}}^{-1}$ E form because the proton spontaneously transferred from the enol to the carboxylate group during the optimization process. (ii) Re-optimization in the gas phase with the proton constrained on the enol function at an O–H distance of 0.97 Å gives a structure of $\text{Pulv2}_{\text{enol}}^{-1}$ that is higher in energy than the $\text{Pulv2}_{\text{COOH}}^{-1}$ form (by $\Delta E = 6.3$ kcal mol⁻¹ at the HF/6-31G(d,p)//HF/6-31G(d) level of theory, and 8.3 kcal mol⁻¹ at the DFT(B3LYP)/6-311G⁺⁺(d,p)//HF/6-31G(d,p) level). Furthermore, when the optimization was pursued after releasing the O–H constraint, the proton jumped back again onto the carboxylate group. (iii) A similar spontaneous transfer was observed in empirical “solution” QM calculations.¹⁹ (iv) A geometry optimization without constraints using a larger basis set (HF/6-31G(d,p)//HF/6-31G(d,p) calculations) confirms that the proton again sits on the carboxylic group, at 1.551 Å from the enolate oxygen, whereas the O–H bond is weakened (0.984 Å). (v) During a Car–Parrinello molecular dynamics simulation of 3.5 ps on the $\text{Pulv2}_{\text{COOH}}^{-1}$ E species, the proton remained on the carboxylate group, without transferring to the enolate oxygen during the dynamics. The average $\text{H}_{\text{COOH}} \cdots \text{O}_{\text{enolate}}$ and (CO)O–H distances are 1.468 ± 0.086 and 1.055 ± 0.029 Å respectively with this method²⁰ (see Fig. S1†). The

Table 1 Total energies (E_{tot} , in Hartree) and relative QM energies (ΔE_{tot} , in kcal mol⁻¹), and NMR chemical shift (in ppm) of the H₈ protons of the different Pulv2, Pulv3 and NBA species (HF/6-31G(d,p)//HF/6-31G(d) calculations)

	Pulv2 ⁰	Pulv2 _{COOH} ⁻¹	Pulv2 _{enol} ⁻¹	Pulv2 ⁻²	Pulv3 ⁰	Pulv3 ⁻¹	NBA _{COOH} ²⁻	NBA ⁴⁻
E_{tot} (Z or Z/Z)	-1290.7382	-1290.2155	-1290.1773	-1289.5400	-1329.7609	-1329.2350	-2457.7598	-2456.2626
E_{tot} (E or E/E)	-1290.7499	-1290.2262	-1290.2162	-1289.5379	-1329.7754	-1329.2407	-2457.7744	-2456.2664
ΔE_{tot} (Z-E)	7.3	6.7	24.4	-1.3	9.1	3.6	-9.2	-2.6
$\delta(\text{H}_8)$ (Z) ^a	7.8	7.6	8.5	8.7	7.7	8.2	7.8	8.8
$\delta(\text{H}_8)$ (E) ^a	7.7	7.6	8.1	8.7	7.4	8.3	7.7	8.9

^a Average over the H_{8a} and H_{8b} *ortho* protons (see Scheme 1 for their definition and Table 4 for their values).

Scheme 1

unusual higher acidity of the enol, compared to the carboxylic acid of the *E* form of Pulv2⁻¹ is due to the stabilization of the enolate function *via* two main contributions: (i) strong internal hydrogen-bonding interaction with the (CO)O–H proton, and (ii) conjugation of the enolate anion with the adjacent groups.²¹

For the *Z* isomer of Pulv2⁻¹, the COOH acidic form is also much more stable than the enol form (by -24.0 kcal mol⁻¹), because the former is stabilized by an internal hydrogen-bond between the (CO)O–H proton and the co-planar lactone oxygen, while the enol is destabilized by the COO⁻ repulsion with that oxygen. As a result, the carboxylate group is co-planar with the lactone ring in the acidic form, and more or less perpendicular to the lactone in the enol form (see structures in Fig. 4 and typical dihedral angles in Table 2). Such internal hydrogen-bonding interactions with COOH as proton donor can be seen in the *Z* forms of Pulv2⁰ and Pulv2_{COOH}⁻¹, and the *E* form of Pulv2_{COOH}⁻¹. The carboxylate group can also be hydrogen-bonded to the H_{enol} proton, *e.g.* in the *E* form of Pulv2⁰ and Pulv2_{enol}⁻¹ (see typical distances in Fig. 4). In the *Z* form of Pulv2_{enol}⁻¹, one can recognize an OH- π -bond interaction between the enol proton and the anisole-2 aromatic ring (see Fig. 4).

None of the studied molecules is fully planar. The anisole-1 ring (see Fig. 2) is co-planar with the 5-membered ring lactone, likely due to C–H...O interactions and π -conjugation, but the anisole-2 ring is tilted by ≈ 30 – 60° with respect to the rest of the molecule. As mentioned above, when it is not involved in an internal H-bonding interaction, the carboxylate group is more or less perpendicular, and thus not conjugated with its adjacent rings.

The COOMe ester group of the Pulv3 molecule cannot act as a proton donor, but can accept a proton from the adjacent OH_{enol} group in the neutral form of the molecule. As a result, the *E* form of Pulv3⁰ is more stable than the *Z* one by 11.2 kcal mol⁻¹. This is higher than the *E*–*Z* energy difference in the Pulv2⁰ analogue, presumably because of the higher basicity of

the carbonyl oxygen in Pulv3⁰. The *E* form of Pulv3⁰ involves a 7-membered ring with an internal hydrogen-bond as in the Pulv2⁰ analogue (see Fig. 4), at an OH_{enol}...O=C distance of 1.660 Å. Interestingly, the deprotonated Pulv3⁻¹ species cannot afford internal hydrogen-bonds, but its *E* form is also more stable than the *Z* one, by 3.6 kcal mol⁻¹.

(2) QM studies on the norbadione molecule

We optimized at both HF and DFT levels (6-31G(d) basis set) the structures of the *E/E* and *Z/Z* isomers of NBA²⁻ and NBA⁴⁻ *in vacuo*, starting from the final structures obtained after 1 ns of classical molecular dynamics simulation in the gas phase. The corresponding energies are given in Table S2† and the HF/6-31G(d,p)//HF/6-31G(d) results are reported in Table 1. Again, the different methods afford similar relative energies, and therefore only the HF/6-31G(d,p) results are discussed in the following.

In the case of the di-deprotonated NBA²⁻ species, the two arms were built with identical protic states, and both enol- and acid-deprotonated *E/E* and *Z/Z* forms were optimized. As in the case of the pulvinic acid, the most stable form is the *E/E* bis-carboxylic acid with two enolate oxygens, stabilized by an intramolecular H-bond in each arm, at short (CO)O–H...O_{enolate} distances (of 1.561 and 1.563 Å respectively), corresponding to elongated O–H bonds (0.981 Å).²² In the most stable *Z/Z* isomer, the proton also strongly prefers to sit on the carboxylic acid group, involved in an intra-arm hydrogen-bond with a O(ring) lactone oxygen.²³ As no such H-bond can form in the *Z/Z*-enol analogue (see Fig. S6),† the latter is markedly less stable than the *Z/Z*-COOH form (by 37.3 kcal mol⁻¹).

In the case of the NBA⁴⁻ species, no intramolecular H-bond can be formed, and the optimized *E/E* form is found to be slightly more stable than the *Z/Z* one, by 2.6 kcal mol⁻¹.

(3) Proton chemical shifts of the *Z* and *E* forms of pulvinic acids and norbadione

The calculated ¹H NMR chemical shifts of Pulv2 and Pulv3 in their neutral and deprotonated states are sensibly the same for the two molecules in a given state (see atom labels and average shifts for H₈, H_{8'}, H₉, H_{9'}, H₁₁, H_{11'} protons in Table 3). Those obtained at the B3LYP/6-311G⁺⁺(d,p) level of calculations are identical within 0.1 to 0.3 ppm to those obtained at the HF/6-31G(d,p) level. In order to be consistent with the NMR results obtained on norbadione, we discuss the HF/6-31G(d,p) results in the following. Concerning the H_{8a} and H_{8b} *ortho*-protons of the anisole-2 ring, unequivalent in a given minimum-energy structure, they

Fig. 4 Snapshots of the QM-optimized neutral and deprotonated Pulv2 and Pulv3 species (HF/6-31G(d) calculations) with typical H-bond distances (in Å).

are equivalent on the NMR timescale due to the fast rotation around the C–anisole bond, and their average values have to be compared for a given *Z* or *E* isomer (see Table 4).²⁴ The same remark applies to the two *ortho* H_{8'} protons of

the anisole-1 group. We note that the calculated chemical shifts δ are of the same order of magnitude as the experimental ones (see Fig. S3).[†] Furthermore, when the pH increases (*i.e.* going from Pulv2^{COOH}^{−1} to Pulv2^{−2}), H₈ becomes

Table 2 HF/6-31G(d)-optimized parameters of the different Pulv2 and Pulv3 species

		Pulv2 ⁰	Pulv2 ^{COOH} ^{−1}	Pulv2 ^{enol} ^{−1}	Pulv2 ^{−2}	Pulv3 ⁰	Pulv3 ^{−1}
ψ^a	<i>Z</i>	8.5	4.3	72.5	81.4	81.3	65.5
	<i>E</i>	3.1	3.3	23.5	85.2	0.6	76.8
d_{O1-CM}^b	<i>Z</i>	3.677	3.632	4.060	4.157	4.154	4.128
	<i>E</i>	3.689	3.674	3.783	4.196	3.617	4.262
d_{O2-CM}^b	<i>Z</i>	5.054	4.788	4.504	4.397	4.562	4.650
	<i>E</i>	5.007	5.063	5.009	4.336	5.018	4.511

^a ψ is the angle (in degrees) between the plane of the lactone group and the carboxylate CO(O) group. ^b d_{O1-CM} and d_{O2-CM} are the distances (in Å) between the O1 and O2 carboxylate oxygens and the center of mass “CM” of the adjacent anisole-2 group (see Fig. 2).

Table 3 Calculated $\delta(^1\text{H})$ NMR chemical shifts of the different Pulv2 (R = H) and Pulv3 (R = Me) species. HF/6-31G(d,p)/HF/6-31G(d) and DFT-B3LYP/6-311G⁺⁺(d,p)/HF/6-31G(d) calculations

		H ₈ ^a	H _{8a} ^a	H ₉ ^a	H _{9a} ^a	H ₁₁	H _{11a}
HF/6-31G(d,p)/HF/6-31G(d)							
Pulv2 ⁰	Z	7.8	8.2	7.2	7.3	3.6	3.4
	E	7.7	8.5	7.2	7.0	3.6	3.3
Pulv2 _{COOH} ⁻¹	Z	7.6	8.7	6.7	6.9	3.4	3.2
	E	7.6	9.0	6.7	7.0	3.4	3.3
Pulv2 _{enol} ^{-1b}	Z	8.5	8.5	6.8	7.0	3.5	3.2
	E	8.1	8.8	6.7	7.1	3.4	3.3
Pulv2 ⁻²	Z	8.7	9.0	6.3	6.6	3.2	3.1
	E	8.7	8.9	6.4	6.6	3.2	3.1
Pulv3 ⁰	Z	7.7	7.7	7.0	7.4	3.6	3.4
	E	7.4	8.4	7.0	7.4	3.6	3.4
Pulv3 ⁻¹	Z	8.2	8.8	6.7	6.9	3.4	3.2
	E	8.3	8.8	6.8	6.9	3.4	3.2
B3LYP/6-311G ⁺⁺ (d,p)/HF/6-31G(d)							
Pulv2 ⁰	Z	7.4	8.2	7.1	7.2	3.8	3.6
	E	7.2	8.3	7.0	7.0	3.8	3.8
Pulv2 _{COOH} ⁻¹	Z	7.1	8.2	6.6	6.9	3.6	3.5
	E	7.4	9.0	6.8	7.0	3.7	3.5
Pulv2 _{enol} ^{-1b}	Z	8.4	8.4	6.8	7.1	3.7	3.3
	E	8.0	8.8	6.8	7.0	3.6	3.4
Pulv2 ⁻²	Z	8.7	9.0	6.3	6.6	3.6	3.3
	E	8.8	9.2	6.5	6.7	3.6	3.4
Pulv3 ⁰	Z	7.4	7.6	6.8	7.4	3.7	3.6
	E	7.1	8.3	6.9	7.4	3.8	3.7
Pulv3 ⁻¹	Z	8.3	8.9	6.8	6.9	3.6	3.5
	E	8.1	8.8	6.9	6.9	3.7	3.4

^a Averages over the two *ortho* positions. ^b Calculated with a constrained O–H enol bond distance of 0.97 Å.

markedly deshielded (by $\Delta\delta \approx 1$ ppm), H₉/H_{9'} and H₁₁/H_{11'} protons are somewhat shielded ($\Delta\delta \approx -0.3$ ppm), whereas H₈ and the CH₃ anisole protons are quasi-unchanged. We thus mainly focus on the H₈ protons for which Kuad *et al.*⁷ observed a marked downfield shift $\Delta\delta$ in both Pulv2 ($\Delta\delta \approx 0.6$ ppm) and Pulv3 ($\Delta\delta \approx 0.25$ ppm) molecules upon full deprotonation of their OH groups, at pH values above 9.5 and 5 respectively.

As seen in Tables 1 and 3, $\delta(\text{H}_8)$ is very sensitive to the protonation state, but not to the *Z* ↔ *E* isomerisation if one considers the most stable forms.²⁵ For Pulv2, at low pH (Pulv2⁰ form), $\delta(\text{H}_8)$ is about 7.8 ppm for both *Z* and *E*

Table 4 Calculated $\delta(^1\text{H})$ NMR chemical shifts of the two *ortho* H₈ protons of the *Z* and *E* Pulv2⁻¹ and Pulv2⁻² species. *Left part of the Table*: obtained on single optimized structures. *Right part of the Table*: obtained from the Boltzman weighted contributions of all rotamers around the C–anisole dihedral angle at 300 K: $\langle \delta(\text{H}_i) \rangle = \sum_i \delta(\text{H}_i) p_i$ (see Figs. S4 and S5)

	Pulv2 ⁻¹	Pulv2 ⁻²	Pulv2 ⁻¹	Pulv2 ⁻²
$\delta(\text{H}_{8a}/\text{H}_{8b})$ Z	9.1/7.7	8.8/8.2	9.3/7.7	9.1/8.3
$\delta(\text{H}_{8a}/\text{H}_{8b})$ E	7.6/7.6	9.1/8.4	7.6/7.6	9.5/8.3
$\langle \delta \rangle$ Z	8.4	8.5	8.5	8.7
$\langle \delta \rangle$ E	7.6	8.7	7.6	8.7

isomers. The first deprotonation of Pulv2 induces a slight upfield shift (to 7.6 ppm for both *E* and *Z* forms of Pulv2_{COOH}⁻¹). Further deprotonation of the acid leads to a marked increase of $\delta(\text{H}_8)$, to 8.7 ppm for both *Z* and *E* conformers. The H₈ protons of Pulv2 are thus deshielded by about 1.0 ppm upon di-deprotonation of the molecule. In the case of Pulv3, the variation of $\delta(\text{H}_8)$ as a function of the pH is similar. In the neutral Pulv3⁰ form, $\delta(\text{H}_8) \approx 7.4$ ppm for the most stable *E* isomer, and 7.7 ppm for the *Z* one. The deprotonation of the enol group causes a deshielding $\Delta\delta(\text{H}_8)$ of 0.6 ppm, to reach a value of *ca.* 8.3 ppm in both *Z* and *E* forms of the Pulv3⁻¹ molecule.

Thus, in contrast to the Kuad *et al.* assumptions,⁷ the increase of $\delta(\text{H}_8)$ upon deprotonation of the Pulv2 and Pulv3 molecules is not correlated to conformational *Z* ↔ *E* isomerization but to other structural changes, namely rotation of the carboxylic and anisole-2 groups in both *Z* and *E* forms, leading to different relationships between the H₈ protons of the anisole-2 group and the carboxylate group. Typical dihedral angles and O_{COO(H)}...anisole-2 distances are given in Table 2. When the carboxylate group is co-planar to the lactone ring, $\delta(\text{H}_8)$ amounts to 7.4 to 7.7 ppm (Tables 1 and 3) whatever the protonation state and the conformation of the rest of the molecule (see the *E* or *Z* forms of Pulv2⁰ and Pulv2_{COOH}⁻¹, and the *E*-form of Pulv3⁰). In those structures, one observes a perpendicular relationship between the COO(H) group and anisole-2, thus perturbing the magnetic field around the H_{8a} and H_{8b} protons. This contrasts with the structures where the H₈ protons are more deshielded ($\delta = 8.3$ to 8.7 ppm) and the COO(H) group is perpendicular to the plane of the molecule (see the *Z* form of Pulv2_{enol}⁻¹, and the *Z* and *E* forms of Pulv2⁻², Pulv3⁻¹). In those cases, the H₈ protons have no specific interactions with the adjacent carboxylate oxygens, reflected by the longer carboxylate...anisole-2 distances compared to the structures with a co-planar carboxylate group.

NMR calculations on the *E/E* and *Z/Z* isomers of norbadiene in its NBA_{COOH}²⁻ and NBA⁴⁻ forms confirm our above conclusions concerning the lack of correlation between *E* ↔ *Z* isomerization and changes in chemical shifts as a function of the pH. The calculated δ results for the protons that have been followed experimentally⁷ are given in Table 5, and the $\delta(\text{H}_8)$ values are in Table 1. First, note that the results obtained at the DFT and HF levels of theory are quite similar. Also, note that the calculated sequence of chemical shifts δ (H₉ < H₃ < H₈ < H₅ ≈ H₇) follows the experimental one for NBA⁴⁻, while there is a H₃/H₈ inversion for NBA²⁻.²⁶ Also, comparing the two arms of NBA that are not symmetrically equivalent, one sees that the corresponding chemical shifts are close for a given type of proton, and differ by at most 0.2 ppm. They show a clear downfield shift of *ca.* 1 ppm for the H₈ protons, and an upfield shift of -0.3 ppm for the H₉ protons upon deprotonation of both *E/E* and *Z/Z* forms of NBA²⁻, as observed in the pulvinic moieties and experimentally (see Fig. S3).[†] For the other types of proton, the calculated $\Delta\delta$ shifts are smaller. Furthermore, if one compares the *E* and *Z* isomers for a given protonation state, one sees that the $\delta(\text{H}_8)$ values are the same within 0.2 ppm.

Table 5 Total QM energies (E_{tot} , in Hartree), and $\delta(^1\text{H})$ NMR chemical shift (in ppm) of selected protons of different NBA species (see Fig. 1 for definitions). HF/6-31G(d,p)//HF/6-31G(d) and DFT-B3LYP/6-31G(d,p)//DFT-B3LYP/6-31G(d) calculations

		E_{tot}	H_3	H_5	H_7	H_8^{1a}	H_9^{1a}	H_8^{2a}	H_9^{2a}
NBA _{COOH} ²⁻	HF/6-31G(d,p)//HF/6-31G(d)								
	Z/Z	-2457.8128	7.2	9.8	9.6	8.0	6.4	7.7	6.7
NBA _{enol} ²⁻	E/E	-2457.8286	7.4	10.0	9.5	7.7	6.6	7.7	6.6
	Z/Z	-2457.7534	7.6	9.0	9.6	8.3	6.6	8.4	7.1
NBA ⁴⁻	Z/Z	-2456.3027	7.4	9.6	9.9	8.7	6.1	8.8	6.1
	E/E	-2456.3067	8.1	10.0	10.5	8.9	6.2	8.9	6.2
NBA _{COOH} ²⁻	B3LYP/6-31G(d,p)//B3LYP/6-31G(d)								
	Z/Z	-2471.8999	7.2	9.9	9.5	7.8	6.3	7.6	6.5
NBA _{enol} ²⁻	E/E	-2471.9178	7.5	10.1	9.5	7.6	6.3	7.5	6.3
	Z/Z	-2471.8437	7.8	8.9	9.8	8.0	6.3	8.2	6.7
NBA ⁴⁻	Z/Z	-2470.3922	7.7	9.6	9.9	8.8	5.7	8.9	5.8
	E/E	-2470.3971	9.1	10.0	10.6	8.9	5.9	8.9	5.9

^a Averages over the two *ortho* positions.

(4) Molecular dynamics studies on norbadione and its Cs⁺ complexes in the gas phase and in methanol solution

Given the importance of deprotonated states of NBA to complex the Cs⁺ cation,⁷ we decided to simulate by MD its NBA²⁻ and NBA⁴⁻ forms, and their binuclear Cs⁺ complexes. In principle, if the MD simulations were long enough (*i.e.* on timescales of milliseconds to seconds), the different conformers of the solute and the complexes could be sampled during the dynamics at 300 K. The present simulations are run for *ca.* 1 to 2 ns, which is demanding of computer time, but not sufficient for that purpose. This is why we will compare different initial conformers, obtained from the dynamics in the gas phase, which will serve as a reference before immersing the solutes in solution. The three simulated forms of norbadione (NBA_{COOH}²⁻, NBA_{enol}²⁻, NBA⁴⁻) are represented in Fig. 1. The di-anion NBA²⁻ was built with two identical -1 charged arms, each of them possessing either one carboxylate plus one enol group (noted NBA_{enol}²⁻), or one enolate plus one carboxylic acid group (NBA_{COOH}²⁻). The NBA_{enol}²⁻ form has been suggested to be predominant at a pH lower than 8 by NMR titration,⁷ whereas the QM calculations on NBA²⁻ and on the pulvinic moiety indicate that the protonated, COOH form is the most stable in the gas phase. In the tetraanion (NBA⁴⁻) both acid and enol groups are depro-

tonated. During the dynamics, most structures generally remained close to their initial potential-energy minimum, and in particular no $Z \leftrightarrow E$ exchange occurred. Insights into energy features are obtained by analyzing the internal energy of the solute E_{solute} , and the interaction energy of the solvent with the solute, noted E_{solv} (see Table 6).²⁷ These are components of the internal energy and of the free energy which also involves solvent-solvent interactions, comprising enthalpic and entropic components as well, unfortunately not easily derived from MD simulations.

The free norbadione

We consider the NBA²⁻ and the NBA⁴⁻ forms of the free norbadione (without the Cs⁺ cation). Each species has been simulated in the gas phase and in methanol, comparing its four isomers (Z/Z, Z/E, E/Z, E/E). For the NBA²⁻ dianion, we consider the enolic NBA_{enol}²⁻ and acidic NBA_{COOH}²⁻ states. The results of energy-component analysis are given in Table 6. In the following, we first describe the main gas-phase results, and then the results in solution.

In the gas phase, during the dynamics of NBA²⁻, independently of the starting conformation, the two arms of the molecule became perpendicular to the central naphthol ring and fluctuated around the ϕ_1 and ϕ_2 dihedrals (defined in

Table 6 Energy component analysis (in kcal mol⁻¹) of norbadione and its Cs⁺ complexes simulated by MD in the gas phase and in methanol solution: internal energies (E_{solute}) and solute-methanol (E_{solv}) interaction energies. Averages during the last 200 ps of dynamics

		L = NBA _{COOH} ²⁻			L = NBA _{enol} ²⁻			L = NBA ⁴⁻		
		$E_{\text{NBA-Cs}}$	E_{solute}	E_{solv}	$E_{\text{NBA-Cs}}$	E_{solute}	E_{solv}	$E_{\text{NBA-Cs}}$	E_{solute}	E_{solv}
Free L in gas phase	Z/Z	—	83 ± 5	—	—	115 ± 5	—	—	290 ± 5	—
	Z/E	—	81 ± 5	—	—	107 ± 11	—	—	289 ± 5	—
	E/Z	—	79 ± 5	—	—	101 ± 5	—	—	282 ± 5	—
	E/E	—	72 ± 5	—	—	95 ± 5	—	—	274 ± 5	—
Free L in methanol	Z/Z	—	91 ± 10	-366 ± 18	—	126 ± 11	-341 ± 21	—	317 ± 20	-727 ± 21
	E/E	—	86 ± 6	-376 ± 18	—	95 ± 10	-300 ± 22	—	309 ± 21	-743 ± 20
L: 2Cs ⁺ in gas phase	Z/Z	-275 ± 3	-122 ± 5	—	-105 ± 4	-103 ± 6	—	-503 ± 8	-131 ± 5	—
	Z/E	-275 ± 3	-126 ± 5	—	-248 ± 2	-105 ± 5	—	-637 ± 6	-182 ± 5	—
	E/Z	-263 ± 2	-135 ± 6	—	-228 ± 2	-109 ± 7	—	-553 ± 4	-170 ± 5	—
	E/E	-270 ± 2	-147 ± 5	—	-242 ± 4	-99 ± 5	—	-645 ± 7	-185 ± 5	—
L: 2Cs ⁺ in methanol	Z/Z	-137 ± 8	-37 ± 9	-441 ± 22	-139 ± 6	-85 ± 10	-451 ± 22	-462 ± 10	-99 ± 10	-573 ± 21
	Z/E	—	—	—	—	—	—	-536 ± 16	-125 ± 10	-532 ± 21
	E/Z	—	—	—	—	—	—	-522 ± 15	-122 ± 11	-534 ± 20
	E/E	-129 ± 10	-40 ± 10	-441 ± 21	-150 ± 15	-51 ± 10	-391 ± 22	-529 ± 17	-125 ± 11	-533 ± 23

Fig. 1), without undergoing full rotations. For the enol, as well as the COOH form, the sequence of relative stabilities is $E/E > E/Z > Z/E > Z/Z$ ($\Delta E = 0, 6, 13$ and 20 kcal mol^{-1} respectively for $\text{NBA}_{\text{enol}}^{2-}$, and $\Delta E = 0, 7, 9$ and 11 kcal mol^{-1} respectively for $\text{NBA}_{\text{COOH}}^{2-}$), which is fully consistent with the QM results on the pulvinic acid and norbadione. In particular, the most stable isomer of NBA^{2-} is E/E (see Fig. S6)† with the 7-membered ring involving strong H-bonding interactions within each arm (of either $\text{C}-\text{OH}\cdots\text{OOC}$ or $\text{C}-\text{O}\cdots\text{HOOC}$ type). Such interactions cannot occur in the Z/Z isomers that are the least stable. In the Z/E form of $\text{NBA}_{\text{enol}}^{2-}$, however, during the dynamics an H-bond formed between the enolic proton of one arm and the lactone carbonyl oxygen of the other arm, and remained stable until the end of the dynamics. In the corresponding E/Z isomer and in the Z/E isomer of $\text{NBA}_{\text{COOH}}^{2-}$, an H-bond similarly formed between the carboxylate group of one arm and the OH phenolic proton of the other arm.

The fully-deprotonated NBA^{4-} molecule cannot afford internal hydrogen-bonding interactions, but its E/E form is still the lowest in energy, because the repulsion between its two arms is smaller than in the other forms. The stability order of the different isomers is: $E/E > E/Z > Z/E \approx Z/Z$ ($\Delta E = 0, 8$ and 16 kcal mol^{-1}) in the gas phase (see Fig. S7)† for the structures). It is interesting to note that the two arms of the Z/Z form delineate a “cavity” suitable to complex a cation. The three other isomers adopt more “open” conformations with their arms more perpendicular to each other.

We now turn to the methanol solution, where we simulated only the E/E and Z/Z forms of NBA^{2-} and NBA^{4-} . The final structures, shown in Fig. S8,† are close to those in the gas phase. For both NBA^{2-} and NBA^{4-} species, the Z/Z and E/E isomers have quite the same sequence of internal energies as in the gas phase, *i.e.* E/E is intrinsically more stable than Z/Z (by $\Delta E_{\text{solute}} = 31 \text{ kcal mol}^{-1}$ for $\text{NBA}_{\text{enol}}^{2-}$, 5 kcal mol^{-1} for $\text{NBA}_{\text{COOH}}^{2-}$, and 8 kcal mol^{-1} for NBA^{4-} . See Table 6). For both Z/Z and E/E isomers, the $\text{NBA}_{\text{COOH}}^{2-}$ form is markedly intrinsically more stable (by 35 and 10 kcal mol^{-1} respectively), and also interacts better with the solvent (by 25 and 75 kcal mol^{-1} , respectively) than $\text{NBA}_{\text{enol}}^{2-}$. Furthermore, among the Z/Z and E/E isomers of the COOH protonated form, E/E is preferred by the two energy components (by 5 kcal mol^{-1} for the internal energy, and 10 kcal mol^{-1} for the solvation energy), and should be therefore the most populated one in solution.

For the NBA^{4-} species, the E/E isomer should also be more populated than Z/Z in solution because its internal energy is lower (by $\Delta E_{\text{solute}} = 8 \text{ kcal mol}^{-1}$) and because it interacts better with the solvent (by $\Delta E_{\text{solv}} = 16 \text{ kcal mol}^{-1}$). Therefore, there should be no $E \leftrightarrow Z$ isomerization upon deprotonation of NBA^{2-} in solution.

Norbadione complexed with two Cs^+ cations

We then simulated the E/E , E/Z , Z/E and Z/Z isomers of $\text{NBA}_{\text{enol}}^{2-}$, $\text{NBA}_{\text{COOH}}^{2-}$ and NBA^{4-} , initially complexed with two Cs^+ cations, in order to compare different complexation modes. The complexes were model-built with Cs^+ cations close to the oxygen coordination sites of the ligand, in

the “cavity” delineated by the two pulvinic arms. For each isomer we tested several initial positions and first performed 1 ns of dynamics in the gas phase (see final snapshots in Fig. S9).† The final structure of the complex was then immersed in methanol solution and simulated for 1 to 2 ns of dynamics.

In the gas phase, the $\text{NBA}_{\text{enol}}^{2-} 2\text{Cs}^+$ system (see Fig. S9)† formed either an *exo-exo* complex (in the case of the Z/Z isomer) or an *exo-endo* complex (for the three other isomers). In the *exo-exo* complex, both Cs^+ cations are coordinated to the carboxylate group and to the lactone ether oxygen of each arm. In the *exo-endo* complex, one Cs^+ is coordinated as above and the other Cs^+ sits in a pseudo-cavity formed by the carboxylate group of one arm and displays cation- π interactions with one aromatic cycle of the other pulvinic moiety. Concerning the $\text{NBA}_{\text{COOH}}^{2-} 2\text{Cs}^+$ complexes, they all adopt an *exo-exo* mode where the cations interact either with two enolate oxygens or with one enolate and one acid group.²⁸ When these complexes were immersed and simulated in methanol solution, one Cs^+ cation decomplexed from both Z/Z and E/E isomers of the $\text{NBA}_{\text{enol}}^{2-}$ and $\text{NBA}_{\text{COOH}}^{2-}$, due to the competition between the solvent and the ligand. These results are consistent with the NMR titration results which indicate that the norbadione must be tetradeprotonated to complex two Cs^+ cations.⁷ The remaining Cs^+ cation coordinates to NBA^{2-} either *via* the carboxylate group of one arm (E/E conformer of the $\text{NBA}_{\text{enol}}^{2-}$), the enol group of one arm and the ketone group (Z/Z conformers of $\text{NBA}_{\text{enol}}^{2-}$ and $\text{NBA}_{\text{COOH}}^{2-}$), or the acid group of one arm and the enolate group of the other arm (E/E isomer of $\text{NBA}_{\text{COOH}}^{2-}$) (see Fig. 5).

In the case of the tetradeprotonated ligand (NBA^{4-}), the mode of complexation was also studied in the gas phase and in solution, and was also found to be conformer dependent (see Fig. S9)† and Fig. 6). Because the cation coordination mode and ligand conformation turned out to be very similar in the gas phase and in solution, we only discuss the results in solution. In the four examined structures, the two Cs^+ cations remained complexed by the ligand. We first discuss the Z/Z complex where the two Cs^+ cations are *exo*-coordinated and therefore exposed to the solvent. Each Cs^+ coordinates to three oxygens from the same arm of the molecule, *i.e.* to one enolate oxygen and two carboxylate oxygens, completed by methanol oxygens in solution. According to the radial distribution functions (RDFs, see Table S3)†, one finds 5.6 to 5.8 O_{MeOH} oxygens and 2.7–2.8 O_{NBA} oxygens within $4 \text{ \AA}$ of each cation, on average. Note that the O_{MeOH} oxygens are somewhat closer to the cation than those of norbadione, indicating loose contacts between NBA^{4-} and Cs^+ in solution. (Fig. 6).

The Cs^+ coordination in the E/E complex of NBA^{4-} is somewhat different because, contrary to the Z/Z case, the complexation of the two cations is asymmetrical. One Cs^+ (Cs_2) sits in a pseudo-cavity, coordinated to the carbonyl oxygens of both arms and to methanol molecules. This cation is thus more shielded from the solvent than the Cs_1 cation which is partially coordinated to the carboxylate oxygens of one arm, and displays π interactions with the anisole group of the other arm. Cs_2 coordinates 4.8 O_{MeOH} plus 4.4 O_{NBA} oxygens, which confirms that this cation interacts better with NBA^{4-} in the E/E than in the Z/Z form, and is therefore

Fig. 5 Final snapshots of the $\text{NBA}_{\text{enol}}^{2-} 2\text{Cs}^+$ and $\text{NBA}_{\text{COOH}}^{2-} 2\text{Cs}^+$ “complexes” with the *Z/Z* and *E/E* isomers of norbadione simulated by MD in methanol. Orthogonal views (left and middle) and top views (right) involving MeOH molecules within 4 Å of the complexed Cs^+ cation. Note that one Cs^+ (not shown) decomplexed during the dynamics.

somewhat less solvated than Cs_1 which is more exposed to the solvent and coordinates 6.2 O_{MeOH} plus 1.9 O_{NBA} oxygens.

In the *Z/E* form, both cations are complexed by either one enolate (from the *Z* arm) or one carbonyl (from the *E* arm), plus two carboxylate oxygens. Thus, each arm of the NBA^{4-} interacts with one Cs^+ cation whose solvation shell is completed by solvent molecules. A given Cs^+ cation is coordinated to 5.3–5.6 O_{MeOH} and 3.2 O_{NBA} oxygens, on average. In the *E/Z* complex, the environment of the two cations differs, as one cation is complexed by one enol plus one carboxylate group from NBA^{4-} (4 oxygens in total) and the other cation interacts with two carboxylate plus one lactone carbonyl-oxygen. The Cs_1 and Cs_2 cations are additionally coordinated to 5.5 and 6.0 O_{MeOH} and to 3.4 and 3.0 O_{NBA} oxygens, respectively.

The energy-component analysis in terms of the internal energy of the solute E_{solute} , of the solute–solvent interaction energy E_{solv} (Table 6) shows that the two contributions are generally antagonistic. For instance, if one compares the *E/E* and *Z/Z* forms of the $\text{NBA}^{4-} 2\text{Cs}^+$ complex one sees that the former is intrinsically less stable (by 25 kcal mol^{-1}), but it interacts better with the solvent (by 40 kcal mol^{-1}). In the corresponding NBA^{2-} complexes, both *Z/Z* and *E/E* forms have similar internal and solvation energies.

Discussion and conclusion

We have reported QM and classical MD studies on pulvinic acid derivatives and norbadione, with the main aim to gain

insights into their conformation properties and NMR signatures as a function of “the pH” (protonation state). In spite of the possible conjugation of the π -electrons, these molecules are far from being all-planar and display a large number of conformations, involving *Z/E* isomerism around the $\text{C}=\text{C}$ bond that links the lactone ring to the carboxylate and anisole-2 groups. The energy and NMR results provide converging views of the conformation of NBA and of its pulvinic acid constituents, some of which differ from previously reported interpretations of NMR and UV-Vis spectroscopic results.⁷

We first discuss the NMR features, then the energy obtained from QM calculations. Note that the calculated $\delta(\text{H})$ values are obtained in the gas phase, whereas NMR experiments have been carried out in $\text{CD}_3\text{OH}-\text{D}_2\text{O}$ solution in the presence of background electrolyte, and cannot be quantitatively compared. An important computational finding concerns the low sensitivity of the $\delta(\text{H}_8)$ shifts to the *E* $\leftrightarrow$ *Z* isomerization for all studied molecules and protonation states considered (in most cases, the differences in $\delta(\text{H}_8)$ is less than 0.2 ppm).²⁵ This contrasts with the high sensitivity of $\delta(\text{H}_8)$ to changes of the protonation state in some cases, following experimental observations.⁷ In the case of the Pulv2 species, the first deprotonation of Pulv2⁰ to form the most stable Pulv2^{COOH}^{−1} *E* form induces a minor change in $\delta(\text{H}_8)$ (−0.1 ppm), but the second deprotonation to form Pulv2^{−2} induces a marked $\Delta\delta(\text{H}_8)$ downfield shift of 1.1 ppm, and these features are consistent with the experimental observation of a plateau at pH values lower than 8, followed by a downfield shift of ca. 0.6 ppm at higher pH values. For the Pulv3⁰ methylated analogue, the calculated $\Delta\delta(\text{H}_8)$ deshielding upon deprotonation (0.5 to 0.9 ppm, depending on the *Z/E* isomer) is smaller than for Pulv2^{−1}, in keeping with the experimental observation (0.3 ppm).⁷ For norbadione itself, di-deprotonation of NBA^{2-} induces a large $\Delta\delta(\text{H}_8)$ shift of ca. 1.0 ppm for both *Z* and *E* isomers according to the calculations, and of ca. 0.6 ppm according to experimental NMR data. Because similar shifts are obtained for the *Z* and *E* isomers, they cannot be used to discriminate between the latter, in contrast with the assumptions of Kuad *et al.*⁷

The QM calculations on the pulvinic Pulv2 and Pulv3 species predict that in their neutral states they should prefer the *E* form. There is no experimental evidence in solution, but the X-ray structure of Pulv3 has been solved,²⁹ showing that the molecule is also in the *E* form with an internal hydrogen-bond, and the corresponding $\text{O}_{\text{enol}} \cdots \text{O}_{\text{COOMe}}$ distance (2.507 Å) is close to our optimized value (2.587 Å). Concerning Pulv2 in solution at low pH values, the lack of spectral change when the pH increases from 2 to 5 can be interpreted either by the presence of a single Pulv2^{−1} species, or by the evolution from Pulv2⁰ to Pulv2^{−1} retaining the same *E* isomer. Deprotonated states are most relevant in the context of ion complexation, and our calculations fully support the view that Pulv2^{−1} and Pulv3^{−1}, as well as NBA^{2-} , display a marked preference for the *E* form, due to the stabilization brought about by an internal hydrogen-bond, forming a 7-membered ring. The conclusions obtained on NBA^{2-} in the gas phase are strengthened by the MD simulations in methanol solutions, showing that the *E/E* form has the lowest internal energy in solution,

Fig. 6 Final snapshots of the $\text{NBA}^{4-} 2\text{Cs}^+$ complexes with the Z/Z , Z/E , E/Z and E/E isomers of the ligand in methanol. Orthogonal views (left and middle) and top views (right) involving MeOH molecules within 4 Å of the complexed Cs^+ cations.

and that it is better solvated. It should be therefore dominant in solution, as suggested by Kuad *et al.*⁷ There is an interesting difference with the Kuad *et al.* assumptions, however, concerning the proton localization in these species: in all of them, the proton prefers the enolate over the carboxylate oxygen. The higher acidity of the enol, compared to be carboxylic function of norbadione and of pulvinic acid is quite unusual, and stems from the particular constitution and topology of these molecules, discussed above. Further experimental studies (*e.g.* vibrational spectroscopy) on the simulated species and analogues at different pH values should contribute to elucidate this feature.

According to our QM and MD calculations, further deprotonation of NBA^{2-} to form NBA^{4-} should not lead to an isomerization, because both species prefer the E/E form that is intrinsically more stable than the Z/Z one, and is also better solvated by methanol. This conclusion differs from the one of Kuad *et al.*⁷ who assumed that the deprotonation of NBA^{2-} involves an E -to- Z conformational switch. As shown by our NMR calculations (*vide supra*), however, large $\delta(^1\text{H})$ chemical shifts can result from either E or Z forms of pulvinic moieties, and are mainly ascribed to the planar to perpendicular rearrangement of the carboxylate moieties with respect to the lactone plane.

Concerning the Cs^+ complexes of NBA, simulated by MD in methanol solution, no firm conclusion can be drawn on their relative stabilities. For the NBA^{2-} species, however, the simulations indicate that different 1 : 1 $\text{NBA}^{2-} \text{Cs}^+$ complexes can be formed by both E/E and Z/Z isomers of the ligand in its bis-acid or bis-enol forms, or possibly mixtures of both. Furthermore, NBA^{2-} cannot form dicationic complexes, as the latter lose one Cs^+ cation during the dynamics. This contrasts with the fully-deprotonated NBA^{4-} species that can bind two Cs^+ cations in solution, according to our simulations, and in agreement with experimental observations.³⁰ However, we find no evidence concerning the higher stability of Z versus E forms of the NBA^{4-} arms, as Z/Z , Z/E , E/Z and E/E isomers form 1 : 2 complexes that remain associated at the nanosecond timescale. This is also consistent with the interpretation of NMR chemical shifts in terms of a rotation of the carboxylate group, rather than an $E \leftrightarrow Z$ isomerization process.

This paper does not pretend to solve all structural and thermodynamic features involved in the Cs^+ complexation process by norbadione in solution, but hopefully should stimulate further experimental and theoretical studies on this versatile but fascinating system. Beyond analyzing medium and conformational-dependent ligand–cation relationships,

more complex phenomena, like aggregation of these hydrophobic species, should not be overlooked.

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- 18 The rotation barrier between the *Z* and *E* isomers of Pulv2^{−1} and Pulv2^{−2} has been calculated at the HF/6-31G(d) level by stepwise rotating the relevant dihedral angle (from 0 to 180° by increments of 30°), and optimizing all other degrees of freedom.
- 19 The QM studies “in solution” have been performed by immersing the solute “in water”, empirically represented by the polarizable continuum PCM model, as implemented in GAUSSIAN 03 software⁸.
- 20 As expected, the HCOOH...O_{enolate} “hydrogen-bond” vibrates at a lower frequency than the (CO)O–H covalent bond (*ca.* 400 and 2200 cm^{−1} respectively), according to the CP-MD calculations (see Fig. S1†).
- 21 Conjugation of the enolate oxygen with the lactone carbonyl is not sufficient, however, to explain why the enol group is more acidic than the carboxylic one. Indeed, HF/6-31G(d) optimizations on an analogue of Pulv2^{−1} where the lactone carbonyl has been substituted by a methyl group (thereby suppressing the anion conjugation with that carbonyl) show that the proton prefers to sit on the carboxylic acid, instead of the enol group, by 4.4 kcal mol^{−1}. See Fig. S2†.
- 22 Concerning the enol *versus* carboxylic (O)–H form of the *E/E* isomer of NBA^{2−}, both HF and DFT calculations with the 6-31G(d) basis set confirm that the proton prefers to sit on the carboxylic (CO)O–H instead of the enol O–H group. At the HF level, energy minima could be located for both forms, separated by $\Delta E = 12.1$ kcal mol^{−1}. This is *ca.* twice the value found for the *E* isomer of the Pulv3^{−1} moiety. At the DFT level, no energy minimum could be located for the enol from which an (O)–H proton spontaneously transferred to the carboxylic group during the optimization process.
- 23 Concerning the enol *versus* carboxylic (O)–H form of the *Z/Z* isomer of NBA^{2−}, both HF/6-31G(d) and DFT/6-31G(d) levels of calculations confirm that the proton prefers to sit on the carboxylic (CO)O–H instead of the enol O–H group. At both HF and DFT levels, energy minima could be located for the two forms, and these are separated by $\Delta E = 31.2$ and 35.4 kcal mol^{−1} respectively (see Table S2†).
- 24 In principle, the average chemical shifts should be calculated as $\langle \delta_i \rangle = \sum_i p_i \delta_i$, summing over the contribution of all rotamers *i* whose *p_i* population is given by their Boltzmann contribution at 300 K, and δ_i is the corresponding chemical shift. We thus calculated the energy profiles corresponding to rotation around the C–anisole bond of the *Z* and *E* isomers of the Pulv2^{−1} and Pulv2^{−2} species, and the chemical shifts at each step. The corresponding energy curves and $\delta(\text{H}_8)$ shifts are given in Fig. S4 and S5,† and their average values are summarized in Table 4. It can be seen that the values obtained from single optimized structures are the same to within 0.1 ppm as those obtained from the weighted contributions of the rotamers.
- 25 The largest difference $\Delta\delta(\text{H}_8)$ between the *Z* and *E* isomers is found in Pulv2_{enol}^{−1} (0.4 ppm) but, as discussed in the text, this form should not be considered because its O–H proton transfers to the carboxylate group. In the case of the Pulv3⁰ form, $\Delta\delta(\text{H}_8)$ amounts to 0.3 ppm, but the *E* isomer is clearly more stable than the *Z* one (by 11.2 kcal mol^{−1}).
- 26 In fact, $\delta(\text{H}_3)$ is very sensitive to the dihedral angle between the plane of the lactone and the “naphthol ring”, and therefore to the medium, due to possible interactions of enolate oxygens with solvent or cationic species.
- 27 If the MD simulations were long enough, the relative stabilities of the different forms could be inferred from their relative populations, or by performing potential of mean force (PMF) calculations to estimate the change in free energy when one form is changed into another form. These are too time consuming, due to the complexity and numerous degrees of freedom of the systems, especially in the cases of the Cs⁺ complexes, and no firm conclusion can be drawn concerning their relative stabilities that depend on enthalpic, as well as entropic components.
- 28 In the case of the NBA_{COOH}^{2−} 2 Cs⁺ complex, the total energies and the interaction energies between the Cs⁺ cations and the ligand are similar for the different isomers (see Table 6).
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