Ab Initio Study of Nodifloridin-A and Nodifloridin-B in Vacuo and in Solution

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Abstract: - Nodifloridin-A and nodifloridin-B are naturally occurring compounds with similar biological activities. Their molecular structures have similar substituents on a phenol moiety for nodifloridin-A and on a phloroglucinol moiety for nodifloridin-B. An ab initio conformational study in vacuo and in three solvents with different polarities (chloroform, acetonitrile and water) highlights a determining influence of the hydroxybenzene moiety on conformational preferences and energies, largely related to the moiety's number of hydrogen bond donor/acceptor sites. Intramolecular hydrogen bonding is given particular attention in view of its role in influencing conformational preferences and energies and its possible roles in the biological activity.

Key-Words: - 1,3,5-Trihydroxybenzene, Acetonitrile solutions, Acylphloroglucinols, Chloroform solutions, Intramolecular Hydrogen Bonding, Nodifloridin, Phenolic derivatives, Solute-Solvent Interactions, Water solutions.

1 Introduction

Nodifloridin-A and nodifloridin-B are compounds isolated from *Lippia nodiflora* [1], a plant utilized in Chinese traditional medicine to treat swellings and abscesses [2]. They are respectively denoted as Nod-A and Nod-B in this work. Fig.1 shows the two structures [3] and the atom numbering utilized here; only the atoms that are relevant for the analysis and discussion of results are given numbers; atoms in corresponding positions are given the same numbers in the two structures to facilitate comparisons.

Nod-A is a phenol derivative while Nod-B is a phloroglucinol (1,3,5-trihydroxybenzene) derivative [1] pertaining to the large subclass of acylphloroglucinols (APHLG, phloroglucinol derivatives characterised by a COR substituent). The two structures have the same acyl chain, $CO(CH)_6COOH$, at C1 (with three conjugated C=C double bonds, also conjugated to the C7=O14 and C23=O24 double bonds) and the same ether function (OCH_3) at C5. The substituent at C3 (here denoted by R′) differs by the presence of a methyl group at C9 and a double bond between C29 and C30 (conjugated to the C31=O32 double bond) in Nod-A.

The sp^2 O of the acyl chain and an OH *ortho* to it can form an intramolecular hydrogen bond (IHB) that will be termed "first IHB". Additional IHB are made possible by the H-bond donor/acceptor properties of the two COOH. In Nod-B, the presence
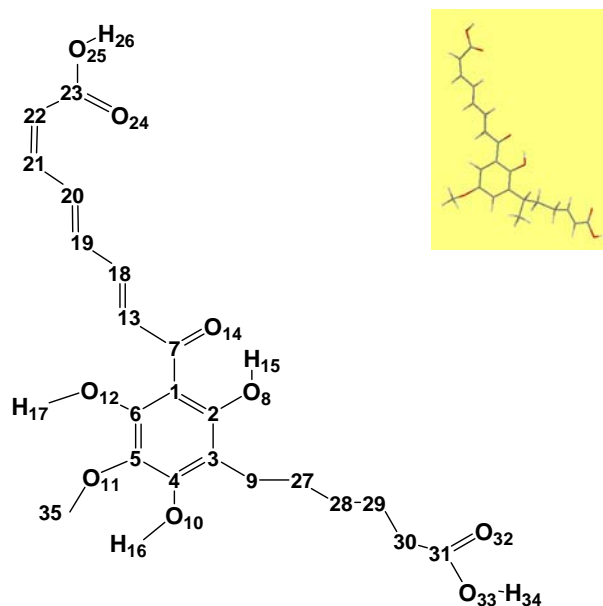

Fig. 1. Structure of Nod-B, showing the atom numbering utilized in this work, and structure of Nod-A (top right corner). The C atoms are indicated only by their numbers.

of the OH group at C6 enables two possibilities for the formation of the first IHB (with H15 or with H17) and the presence of the OH groups at C4 and C6 enables the formation of IHB engaging O11 (with H16 and/or with H17). The study of IHB is particularly important because of their roles in determining conformational preferences and

energies, as well as in the mechanisms of biological activities. In the case of Nod-A and Nod-B, the study of IHB patterns is also important to highlight differences related to the different hydroxybenzenes moieties.

2   Problem Formulation

The interest of a computational study of Nod-A and Nod-B goes beyond the obtainment of information on their individual conformational preferences and on relevant geometry characteristics like the parameters of their IHB. The results enable a range of comparisons, in turn expected to enable better insight on the molecular properties of APHLG and on how APHLG compare with similar derivatives of other phenols. The fact that the two longer chains end with COOH groups (groups capable of forming IHB) further expands the comparison scope.

The following issues are given particular attention in the analysis and discussion of computational results: • the effects, on the characteristics of the first IHB, of the presence of a conjugated π system in the acyl chain, and of the presence of a bulky chain at C3; • the effects of additional IHB, and the competition between the stabilizing effect of an IHB and the geometry constrains that may make the formation of some IHB energetically unfavourable; • comparison with the predictions from simpler model structures, to assess their predictive abilities, and comparison with the results of previous extensive studies of the characteristics of APHLG [4,5]; • comparison of conformational preferences in vacuo and in solution (results in solution being particularly important for biologically active molecules, because their activity within living organisms is exerted in a medium [6]); • comparison of the effects of the presence of three equally-spaced phenol OH in phloroglucinol derivatives, with respect to analogous derivatives of other hydroxybenzenes.

3   Problem Solution

3.1   Computational details

Calculations were performed using the GAUSSIAN 03 package, version D.01 [7].Given the size of the two molecules, and the additional demands of PCM optimisation, all the calculations were performed at the HF/6-31G(d,p) level, because of affordability reasons. Previous comparisons of HF/6-31G(d,p) results with those of higher levels of theory (mostly using MP2/6-31++G(d,p) results as benchmarks, as the best for H-bonding description because of including correlation [8] and dispersion [9] effects

and diffuse functions in the basis set [10,11]) had shown the reasonability of HF/6-31G(d,p) results, above all for the identification of trends, including conformers' relative energies, effects of molecular geometry features on the characteristics of the first IHB, geometry and energy effects of the IHB removal and stabilization patterns in solution [4,5]).

Calculations in solution were performed with the polarisable continuum model (PCM [12,13]), with the   default settings of Gaussian03, considering three solvents with different polarities and different H-bonding abilities (chloroform, acetonitrile and water) to cover the range of media that may be interesting for biological activity [6]. The *in-vacuo*-optimized geometries were utilized as inputs; re-optimization in solution (enabling better quality of the solvation phenomenon description [14]) was performed for the lowest energy conformers; single-point calculations were performed for all the conformers (a study of APHLG had shown that the trends identified from full re-optimization and from single-point calculations in solution are identical, and the energies differ by less than 1.00 kcal/mol).

Adducts with explicit water molecules were calculated because of their significance in the interpretation of PCM results when the solvent molecules can form intermolecular H-bonds with the solute molecules. The interaction energy ($\Delta E_{nod\text{-}aq}$) of an adduct with n water molecules is calculated as

$$\Delta E_{nod\text{-}aq} = E_{adduct} - (E_{nod} + n\, E_{aq}) - E_{aq\text{-}aq} \qquad (1)$$

where E_{nod} is the energy of the isolated solute molecule, E_{adduct} is the energy of the adduct, E_{aq} is the energy of an isolated water molecule and $E_{aq\text{-}aq}$ is the overall interaction energy between water molecules (evaluated through a single-point calculation on a cluster of sole water molecules arranged in the same way as in the adduct [15]). Both E_{adduct} and $E_{aq\text{-}aq}$ are corrected for BSSE using the counterpoise method [16].

The stability of the anions (an acidity determining factor) was estimated through the strength of its H-bonds with water molecules attached to the COO⁻ groups; in this case, the 6-31+G(d,p) basis set was utilized because of the relevance of diffuse orbitals for the study of anionic species [14].

All the energy values reported are in kcal/mol and all the distances are in angstrom (Å). For conciseness sake, the media are denoted with acronyms on reporting values: vac (*vacuum*), chlrf (chloroform), actn (acetonitrile) and aq (water).

3.2   Results of the calculations *in vacuo*
3.2.1   Conformational preferences
Conformers are here named systematically. The

names start with A for Nod-A and B for Nod-B. Relevant geometry features are denoted with symbols (table 1). For each structure, conformers with different geometries of the acyl chain and/or R′ are numbered in order of increasing energy, and conformers that differ only by the features denoted by symbols (table 1) are given the same number. Only conformers with Z form of the COOH groups were calculated, as this is known to be the more stable form of the carboxyl function.

Table 2 reports the relative energies of the best conformers of Nod-B (considering all the media) and Fig.2 shows the preferred geometries *in vacuo*, whose corresponding conformers (B-1-s-w-aj, B-1-s-w-aj-t, B-2-s-w-bfj, B-2-s-w-bfj-t, B-3-s-w-afj and B-3-s-w-afj-t) account for practically all the population *in vacuo*. These conformers combine the highest number of stabilizing effects: the presence of the first IHB; additional IHB involving the COOH, without excessive disruption of the planarity of the acyl chain (important for conjugation stabilization); the H16···O11 IHB and uniform orientation of the three phenolic OH [4,17]. When there are no IHB involving the COOH functions (higher energy conformers), d-w options become the preferred ones because of the possibility of two IHB with O11.

The lowest energy conformer of Nod-A (A-1-d-t) corresponds to the geometry shown in fig.1, and the other populated conformers differ by features like the orientation of the ether methyl group or of R′ or some torsion angles in R′. Table 3 reports the relative energies of representative conformers.

The results of both structures highlight the

S	IHB present [1]	S	geometrical feature
d	H15···O14	r	H16 is oriented toward R′
s	H17···O14	w	H16 is oriented toward the ether function
a	H15···O32		
b	H15···O33	u	H15 or H16, not engaged in the first IHB, is oriented "upward" [2]
c	H16···O32		
e	H16···O33	p	when not engaged in IHB, O24 is oriented upward [2]
f	H26···O32		
g	H26···O33	q	when not engaged in IHB, O32 and O33H34 are oriented oppositely to the case without this symbol
h	H26···O12		
j	H34···O24		
k	H34···O8	t	the methyl of the ether function is oriented "towards us" with respect to the plane of the benzene ring [2,3]
m	H34···O10		
n	H34···O25		

Table 1. Symbols (S) utilized to denote the main geometry features of the conformers.
[1] No symbol is introduced for the IHB with O11 in Nod-B, because their presence or absence is clear from other features: all w conformers have the H16···O11 IHB and all conformers that are not s-type or d-u-type have the H17···O11 IHB; [2] The way in which the structures are reported in fig. 1 is taken as reference; [3] If there is no indication in the name, the orientation is opposite to the one identified by this symbol.

conformer	relative energy			
	vac	chlrf	actn	aq
B-1-s-w-aj	0.000	0.000	0.000	5.283
B-2-s-w-bfj	0.438	1.650	2.233	9.842
B-2-s-w-afj	0.935	2.093	2.691	10.337
B-3-d-r-c	2.967	1.032	0.460	2.697
B-5-d-w	4.373	1.176	0.076	0.000
B-2-s-w-afj-u	4.532	5.268	5.592	10.902
B-7-d-w	5.240	2.104	1.088	1.151
B-3-d-r-cp	4.692	2.451	1.832	3.914
B-5-d-r	6.744	3.668	2.490	2.098
B-5-s-w	7.116	3.988	2.814	2.184

Table 2. Relative energies of selected conformers of Nod-B in different media. The results reported for the three solvents correspond to full re-optimization in solution.
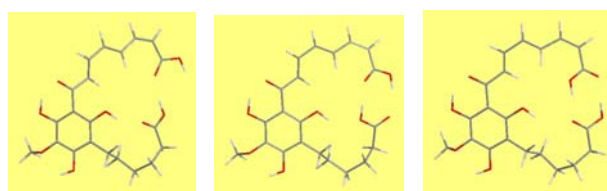

Fig. 2. Geometries of the lowest energy conformers of Nod-B *in vacuo*: from the left: B-1-s-w-aj, B-2-s-w-bfj and B-3-s-w-afj.

conformer	relative energy			
	vac	chlrf	actn	aq
A-1-d-t	0.000	0.000	0.000	0.000
A-1-d	0.079	0.072	0.014	0.039
A-2-d	0.244	0.252	0.259	0.273
A-4-d	0.990	0.754	0.693	0.611
A-5-d	1.383	1.192	1.227	1.183
A-6-fj	5.828	8.200	8.913	–
A-7-f	8.158	14.47	–	–

Table 3. Relative energies (kcal/mol) of selected conformers of Nod-A in different media. The results reported for the three solvents correspond to full re-optimization in solution.

dominant stabilizing effect of the first IHB. In Nod-B, where the presence of O12H17 enables the formation of the first IHB on the left, the lowest energy conformers have both the first IHB and additional IHB engaging H15 and the two COOH. In Nod-A, where the formation of IHB involving H15 and the COOH would prevent the formation of the first IHB, the best conformers have only the first IHB (the relative energy of the lowest-energy conformer with IHB involving the COOH groups, A-6-fj, is 5.828, what makes it unpopulated).

Not all the possible IHB have a stabilizing effect. This can be ascribed to a greater weight of the destabilization due to geometry strains with respect to the stabilization associated with the IHB, for certain IHB; the issue will be discussed more in detail on considering the IHB energy (section 3.2.3).

Other geometry features may have non-negligible influence on the conformers' energy. When not

engaged in IHB, the COOH group of the acyl chain prefers an orientation like the one shown in fig.1, with the OH "up"; the reverse orientation (with the OH "down") causes a 1.72−1.80 energy increase in Nod-B and 1.87−1.89 in Nod-A. The influence of the orientation of the COOH of R′ (when not engaged in IHB) is considerably less; e.g., it is 0.76 for B-d-w-1 and 0.42 for A-d-1. The orientation of the methyl of the ether function with respect to the plane of the benzene ring does not cause significant energy differences: 0.011−0.374 for Nod-B and 0.059−0.186 for Nod-A. The R and S conformations for the chiral C9 atom in Nod-A differ by 0.054−0.614. It is not possible to single out the effect of the uniform orientation of the phenol OH in Nod-B because changes in the orientation of any of the three OH implies formation or removal of some IHB; however, previous studies [4] show consistency among acylphloroglucinol, enabling to consider it as ≈1.

3.2.2 Characteristics of the IHB

Table 4 reports the parameters of the IHB present in the lowest energy conformers of Nod-B, and in other conformers representative of different IHB combinations (selecting the lowest energy one for each combination). The IHB engaging O11 (H16···O11 and H17···O11) are not included because the regularity of their trends and geometry (due to the constraints from the benzene ring) makes a discussion in the text adequately informative.

The first IHB is always the shortest IHB present in a conformer. Its length shows trends similar to those identified for APHLG [4]: shorter when it forms on the same side as R′ (1.670−1.706/d-w and 1.684−1.692/d-r) and longer when it forms on the other side (1.710−1.763/s-w and 1.726−1.767/s-r; always longer for s-r for corresponding s-w/s-r pairs). Comparison of the lowest energy conformers of Nod-B without additional IHB (B-5-d-w) with the corresponding conformers of structure MB-1 (fig.3; an actual acylphloroglucinol [1] and a suitable model for Nod-B) shows no difference in the first IHB length for d-r and d-w cases, whereas the IHB is longer in Nod-B for s-r and s-w cases; this suggests that a methyl at C3 is a good model for the bulkier R′ and for the combined effects of the acyl chain and R′, while a COCH$_3$ group may not be adequate to model a longer acyl chain containing a conjugated π system (whose effect is the only effect on the O14··· H17 IHB). A model structure with the same acyl chain, a methyl at C3 and no ether function at C5 gives closer values (less than 0.004 difference) for the s-w and s-r first IHB, but not as

conformer and IHB	H-bond parameters			ΔE
	O····H Å	O····O Å	OĤO	
B-1-s-w-aj	1.747	2.575	143.0	0.000
H15···O32	1.953	2.850	157.1	
H34···O24	1.882	2.823	166.9	
B-2-s-w-bfj	1.710	2.545	143.8	0.438
H15···O33	2.281	3.102	145.0	
H26···O32	1.968	2.894	161.8	
H34···O24	1.744	2.699	170.1	
B-2-s-w-afj	1.722	2.556	143.6	0.935
H15···O32	2.551	3.416	152.3	
H26···O32	1.850	2.793	167.1	
H34···O24	1.814	2.777	178.8	
B-3-d-r-c	1.684	2.531	145.3	2.967
H16···O32	1.991	2.888	157.3	
B-4-s-w-a	1.728	2.559	143.4	3.759
H15···O32	2.002	2.888	155.0	
B-5-d-w	1.699	2.540	144.6	4.373
B-8-d-w	1.704	2.544	144.5	5.547
B-9-s-w-f	1.763	2.591	143.1	5.835
H26···O32	1.888	2.839	172.7	
B-10-d-w-m	1.700	2.541	144.7	6.130
H34···O10	2.460	3.100	124.7	
B-5-d-r	1.687	2.531	144.9	6.744
B-11-s-w-b	1.750	2.575	142.7	7.049
H15···O33	2.271	2.988	132.2	
B-5-s-w	1.725	2.556	143.3	7.116
B-1-s-r-aj	1.767	2.591	142.7	7.509
H15···O32	1.942	2.851	159.9	
H34···O24	1.868	2.812	167.6	
B-13-s-w-agn	1.762	2.587	142.6	7.993
H15···O32	1.996	2.870	152.7	
H26···O33	2.545	2.958	106.4	
H34···O25	2.469	2.958	111.9	
B-15-s-w-bfn	1.760	2.585	142.6	8.373
H15···O33	2.371	3.111	135.1	
H26···O32	1.988	2.832	146.1	
H34···O25	2.503	3.333	145.8	
B-2-s-r-bfj	1.726	2.557	143.4	8.584
H15···O33	2.283	3.121	147.5	
H26···O32	1.979	2.905	162.0	
H34···O24	1.730	2.688	170.9	
B-12-d-r-e	1.691	2.534	144.9	8.801
H16···O33	2.627	3.221	121.3	
H34···O10	2.895	3.221	101.4	
B-10-s-w-m	1.731	2.561	143.1	9.212
H34···O10	2.409	3.088	128.4	
B-4-s-w-q	1.714	2.549	143.9	10.365
H15···O32	2.004	2.906	158.7	
B-16-d-w-k	1.670	2.521	145.6	11.371
H34···O8	2.285	3.157	152.8	
B-17-s-w-agq	1.739	2.568	143.1	11.640
H15···O32	2.076	2.970	157.0	
H26···O33	2.047	2.966	162.1	
B-3-s-r-c	1.745	2.573	143.2	11.996
H16···O32	1.989	2.895	159.7	
B-19-d-w-h-q	1.746	2.577	143.7	14.226
H17···O25	2.608	3.094	112.2	
H26···O12	2.926	3.094	91.2	
B-5-s-r	1.746	2.572	143.0	15.616

Table 4. Parameters of the IHB in representative conformers of Nod-B, *in vacuo*.
The values in the row with the name of the conformer correspond to the first IHB. The other IHB present in the given conformer are listed below its name. The conformer relative energy (ΔE) is included as reference for the the stabilizing effect of each set of IHB in association with the corresponding geometry.
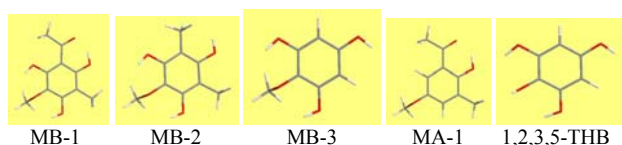
MB-1   MB-2   MB-3   MA-1   1,2,3,5-THB

Fig. 3. Structures utilized as simplified models or for other comparisons.

close for the d-r and d-w cases.
The length of H16⋯O11 and H17⋯O11 depends on whether they are present simultaneously or singly. H16⋯O11 is 2.111−2.117 when it is alone (s-w), with slight discrepancies only in conformers with relative energy >7.1, and 2.201−2.243 when H17⋯O11 is also present (d-w, w); H17⋯O11 is 2.057−2.068 when it is alone (d-r, r), with slight discrepancies only in conformers with relative energy >8.8, and 2.154−2.262 when H16⋯O11 is also present (d-w, w). When they are both present, H17⋯O11 is 0.016−0.084 shorter than H16⋯O11 if the first IHB is also present, 0.07−0.033 longer if the first IHB is absent. Comparison with a conformer of 1,2,3,5-trihydroxybenzene having bifurcated IHB (1,2,3,5-THB, fig.3) shows close values for the IHB lengths when H16⋯O11 and H17⋯O11 are present simultaneously.

The IHB involving one or both COOH groups shows a variety of ranges, and their length appears to depend considerably on the molecular context. E.g., H15⋯O32 (IHB between a phenol OH and a carboxyl sp^2 O) is shortest in B-1-s-r-aj and longest in B-12-s-w-agn, with an even longer value in B-2-s-w-afj, where O32 is engaged in a bifurcated IHB. Of the IHB between COOH groups, the shortest are (predictably) those between the OH of one group and the sp^2 O of the other, whereas IHB engaging the two carboxyl OH are longer.

The length of the first IHB in Nod-A (1.739−1.766) is closer to the first IHB of Nod-B engaging H17 than to that engaging H15. The lengths of the other IHB (appearing only in high energy conformers) fall within the ranges observed for nod-B; e.g., the range for H26⋯O32 is 1.923−1.964. For both structures, the IHB parameters are not influenced significantly by the orientation of the ether methyl with respect to the plane of the ring.

3.2.3 The strength of the IHB
The evaluation of IHB energy is a difficult task, because there is no suitable reference [18]. The removal of an IHB by 180° rotation of the donor OH leaves the lone pairs of the two O atoms exposed to mutual repulsion and may cause geometry changes in the rest of the molecule; both factors (geometry changes and O↔O repulsion) contribute to the

energy difference between the conformers with and without the IHB, preventing the isolation of the effect of the IHB removal.

For the first IHB in APHLG, the O↔O repulsion is partially smoothed by the off-plane shift of the acyl group on IHB removal [4,5] and there are no other relevant geometry changes; therefore, the energy difference between the conformers with and without the IHB can be viewed as a reasonable indication of the IHB energy. The shift occurs also in Nod-B, bringing O14 51−54° off-plane (63−74° in APHLG with no π systems in the acyl chain conjugated to C7=O14, 51−54° in the few calculated structures with a π systems conjugated to C7=O14 [4,5]). In all APHLG with R′≠H, the IHB on the same side as R′ is stronger than when it forms on the other side [4]. The energy increase on its removal from d-r or d-w Nod-B conformers (11.56−13.43; 11.63 for the removal from B-d-w-5) is comparable with that of the other APHLG (8.72−12.10). For the first IHB engaging H17, an independent evaluation is impossible, as its removal brings the formation of the H17⋯O11 IHB; the IHB length suggests that H17⋯O14 is somewhat weaker than H15⋯O14, consistently with the results for all other APHLG with R′≠H [4,5].

On removing the first IHB from Nod-A, the O↔O repulsion can disappear completely through simultaneous 180° rotations of H15 and of O14. The energy increase on H15⋯O14 removal from A-1-d-t is 8.454. In general, the energy increase on removing H15⋯O14 is 3.105−4.978 smaller for Nod-A than for nod-B, due to the combined effects of the absence of overestimations associated with O↔O repulsion and of H15⋯O14 being somewhat weaker in Nod-A, as also suggested by its greater length.

Fig.4 highlights the different patterns for APHLG and for acylated phenol derivatives, through the scans of the rotations of relevant bonds in model structures: MB-1 for Nod-B and MA-1 for Nod-A (fig.3). The scan of H15 shows the barrier for IHB removal by 180° rotation of the donor. The MB-1 pattern is consistent with that of other APHLG; e.g., the barrier is 12.975 (11.400−13.491 for APHLG) and O14 moves steadily away from O8 as the IHB weakens, reaching 65° off-plane (as typical for APHLG in IHB absence). The MA-1 pattern differs by a partial "return" of O14 towards O8 (returning to 54° in the last steps after reaching 61° off-plane for 150° torsion angle of H15, with a 13.146 barrier). Although the height of the barrier can be an indicator of the IHB strength [19], the two patterns show that, in the case of hydroxybenzenes
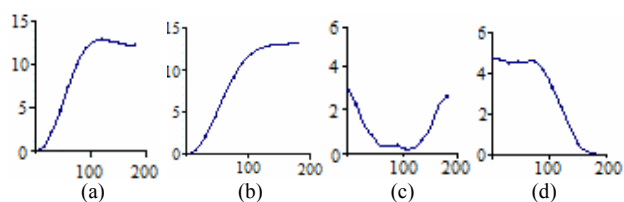
Fig.4. Scans of the rotation of H15 (energy versus C1C2O8H15 torsion angle, removing the IHB) for MB-1 (a) and MA-1 (b), and scans of the rotation of O14 (energy versus C2C1C7O14 torsion angle, in absence of IHB) for MB-1 (c) and MA-1 (d).

derivatives, other factors (besides the IHB removal) contribute to the height: the residual O14↔O8 repulsion (decreased, but not removed by the off-plane shift of O14) and the destabilization associated with the off-plane orientation of O14. A comparison with the energy increase on IHB removal for nod-A (no O14↔O8 repulsion in the conformer without the IHB) suggests that the overestimation of the IHB energy due to these factors may be in the 2.7–4.7 range (2.6─5.2 from estimations on APHLG [4,20]).

The scan of the rotation of O14 in conformers without IHB shows the low-energy orientations of O14 when not engaged in the IHB: two minima, at 60° off-plane from O8 or O12, for MB-1 (balancing the tendency to decrease O↔O repulsion and the tendency to avoid perpendicular orientation, like the other APHLG [4]) and on the other side with respect to O8 for MA-1, with disappearance of the O↔O repulsion and additional stabilisation from an unconventional H-bond with the H at C6.

Estimating the energy of the IHB involving atoms of the COOH groups is practically impossible, because of the dramatic geometry changes on removing them. Comparisons of pairs of conformers in which one of these IHB is respectively present and absent (table 4) highlight the great weight of geometry factors: in a number of cases, the conformer without a given IHB has better energy than the conformer with that IHB, showing that the energy-increasing effects of the geometry strains in one or both chains overcome the stabilizing effect of the IHB. Because of the impossibility of separating the two types of effects, it is considered that the best option for a first-approximation comparison of the IHB strength is by comparing their lengths (the IHB length being a fair indicator of its strength).

The energy of H16···O11 in Nod-B can be estimated from comparison of w-r pairs. Using a rough ~1.0 estimation [4,17] for the stabilization associated with uniform orientation of the three phenol OH in d-r and s-w forms, its energy is 6.5─7.1 when it is on the same side as the first IHB (s forms) and 3.0─3.4 when it is on the other side (d

forms). Estimating the energy of H17···O11 is complicated by the fact that its removal results in u-type conformers, with considerable energy increase from steric effects [4]. Estimations on the simplest phloroglucinol with an ether function (MB-3, fig.3) give 2.7 for the removal of one IHB and 7.2 for the simultaneous removal of both. Estimations on model structure MB-2 (mimicking the steric effects at C1 and C3 by two methyls to remove the complications of u-type geometries with a carbonyl) give 4.6 for the removal of one IHB and 9.9 for the removal of both. These values are very close to those for the bifurcated IHB in 1,2,3,5-THB, suggesting that an ether O and a phenol O may have very close H-bond acceptor ability when attached to a benzene ring and engaged in IHB closing a 5-member ring.

3.4 The dimers

The H-bonding ability of Z-form COOH functions enables the formation of dimers held together by two inter-monomer H-bonds. The presence of two COOH in Nod-A and Nod-B enables the formation of three types of dimers, depending on the COOH functions involved: C23O24O25H26 in both monomers, C23O24O25H26 in one and C31O32O33H34 in the other, or C31O32O33H34 in both; they are here respectively denoted by aa, ab and bb. In aa cases, both COOH are at the end of a π conjugated system; in ab cases, only one COOH is at the end of a π conjugated system; in bb cases, R′ has no double bond conjugated to COOH for Nod-B, while there is one for Nod-A. Fig.5 shows the dimers of the lowest energy conformer of Nod-B with both COOH not engaged in IHB (B-5-d-w); table 5 reports the relative energy, the interaction energy between the two monomers and the parameters of the H-bonds between the two monomers for the dimers of B-5-d-w and for the dimers of the lowest energy conformer of Nod-A (A-1-d-t). The values show no difference that can be ascribed to the hydroxybenzene moiety and no significant influence from the rest of the chain to which the COOH are attached, suggesting that the dominant factor is the H-bonding ability of the COOH groups.
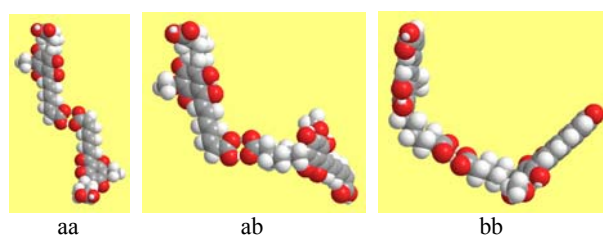

Fig.5. The three dimers consisting of two B-5-d-w monomers.

dimer	ΔE	ΔE_{dim}	parameters of the H-bonds		
			O···H Å	O···O Å	OĤO
B-5-d-w dimer bb	0.000	13.109	1.823 1.823	2.783 2.783	176.2 176.2
B-5-d-w dimer ab	0.067	13.047	1.811 1.830	2.772 2.890	176.7 176.2
B-5-d-w dimer aa	0.187	12.921	1.818 1.818	2.779 2.779	176.8 176.8
A-1-d-t dimer bb	0.000	13.432	1.807 1.807	2.769 2.769	176.6 176.6
A-1-d-t dimer ab	0.128	13.304	1.788 1.836	2.752 2.795	177.1 176.2
A-1-d-t dimer aa	0.557	12.876	1.819 1.819	2.779 2.779	176.7 176.7

Table 5. Relative energy (ΔE, kcal/mol), interaction energy between the two monomers (ΔE_{dim}, kcal/mol, corrected for BSSE) and parameters of the H-bonds between the two monomers, for the dimers of B-5-d-w and of A-1-d-t.

3.5 Results of PCM calculations in solution

The results in solution show different patterns for different solvents. The polarity of the solvent molecules and their ability to form intermolecular H-bonds with the solute molecules play major roles in determining solute conformational preferences. In the PCM model, the bulk effect of the solvent polarity is described by the dielectric constant (4.90/chlrf, 36.64/actn, 78.39/aq). Although PCM does not take solute-solvent H-bonds into explicit account, the relative energies resulting from the bulk consideration of solute-solvent interactions indicate preferences for the presence or absence of a certain IHB in solution, thus suggesting which IHB would likely break in a given solvent.

The polarity influence is particularly evident in the case of Nod-B, where conformers with several IHB are preferred *in vacuo*, in chloroform and in acetonitrile (table 2; the lowest energy conformer is the same in vac, chlrf and actn and the relative energy pattern of the other conformers is the same in chlrf and actn), while the lowest energy conformers in water solution have only the first IHB, leaving the two COOH functions available for intermolecular H-bonds. This is consistent with the results for APHLG, showing that the first IHB does not break in aq solution on competition with the possibility of formation of intermolecular H-bonds, and with the results for caespitate [20], showing that the weaker IHB between a phenol OH and an ester sp^2 O in R′ breaks on such competition. The fact that IHB between the two COOH in Nod-B (stronger than an IHB with an ester O) are not favoured in water solution supports the hypothesis that only the first IHB of APHLG does not break in water solution.

The solvent polarity influences also the IHB

parameters. For Nod-B, the length of the first IHB, and of H16···O11 and H17···O11, increases with increase in solvent polarity, while that of H16···O32 decreases. The first IHB length increases by 0.001-0.002/chlrf, 0.002-0.006/actn, 0.032-0.039/aq for s-w cases with IHB between COOH also present, by 0.004-0.010/chlrf, 0.007-0.013/actn 0.039-0.054/aq for d cases. The H17···O11 length increases by 0.022-0.029/chlrf, 0.028-0.043/actn, 0.090-0.122/aq. The H16···O11 length increases by ~0.026/chlrf, 0.038-0.040/actn, 0.098-0.102/aq when it is alone in s-w cases, by considerably less (0.013-0.022/chlrf, ~0.020/actn, ~0.035/aq) when H17···O11 is also present. The changes in the length of the IHB between the two COOH (different for H26···O32 or H34···O24) depend on the accessibility of the IHB by the solvent molecules and on the solvent polarity. For the conformers of Nod-A without additional IHB, the length of the first IHB decreases by 0.006-0.014/chlrf, 0.010-0.012/actn, 0.001-0.010 /aq.

The estimation of IHB strength in solution is complicated by the greater stabilization, by the solvent, of conformers without IHB with respect to those with the IHB, preventing the possibility of considering their energy difference as an approximation to the IHB energy [5]; the IHB length remains a reasonable indicator of the IHB strength.

Table 6-a reports the solvent effect (free energy of solvation, ΔG_{solv}) for selected conformers, from calculations with full re-optimization in solution, and Table 6-b reports the corresponding electrostatic (G_{el}) and non-electrostatic (G_{non-el}) contributions.

The single-point calculations in solution offer a complete picture because of having been performed for all the conformers. ΔG_{solv} is always negative for aq, always positive for actn, and mostly negative for chlrf. The values of ΔG_{solv} have the broadest range in aq solution, with clear correspondence to the number of free H-bond donor-acceptor sites. For Nod-B (considering ΔG_{solv} absolute values and single-point results): ~2.4 with no site available (e.g., B-2-s-w-bfj); ~6−7 with one site of a COOH available (e.g., B-1-s-w-aj); ~14−15 with one COOH completely available and the other partially available (e.g., B-3-d-r-c); ~17−19 with both COOH completely available (e.g., B-5-d-w) and ~20−25 for conformers with no IHB. For Nod-A: ~5 with no site available (e.g., A-6-fj), ~8 with one site of a COOH available (e.g., A-7-f), ~16 with both COOH completely available and ~18 for conformers with no IHB (some absolute values being greater for Nod-B than for Nod-A, because of the higher number of H-bond donor/acceptor sites).

#	conformer	ΔG$_{solv}$		
		chlrf	actn	aq
1	B-1-s-w-aj	1.23	6.83	−8.58
2	B-2-s-w-bfj	2.82	9.07	−3.66
3	B-2-s-w-afj	2.91	9.20	−3.49
4	B-3-d-r-c	−2.26	2.97	−16.14
5	B-5-d-w	−3.64	1.12	−20.65
6	B-2-s-w-afj-u	2.60	8.53	−6.73
7	B-7-d-w	−3.42	1.41	−20.12
8	B-3-d-r-cp	−2.59	2.58	−16.73
9	B-5-d-r	−3.60	1.06	−21.10
10	B-5-s-w	−3.63	1.00	−21.78
11	A-1-d-t	−2.90	2.35	−17.19
12	A-1-d	−2.88	2.30	−17.23
13	A-2-d	−2.33	2.88	−16.49
14	A-4-d	−3.19	2.00	−17.59

Table 6-a. Solvation free energy (ΔG$_{solv}$), for selected conformers of Nod-B and Nod-A.
All the results correspond to full re-optimization in solution. The numbers in the first column are introduced to serve as references for table-6-b.

#	G$_{el}$			G$_{non-el}$		
	chlrf	actn	aq	chlrf	actn	aq
1	−5.73	−8.39	−19.85	6.96	15.22	11.27
2	−4.47	−6.49	−15.49	7.29	15.56	11.83
3	−4.51	−6.50	−15.39	7.42	15.70	11.89
4	−7.68	−10.89	−25.50	5.42	13.85	9.36
5	−8.93	−12.67	−29.68	5.29	13.79	9.04
6	−5.01	−7.37	−18.88	7.62	15.90	12.15
7	−8.86	−12.50	−29.36	5.44	13.91	9.24
8	−8.00	−11.26	−26.07	5.40	13.83	9.34
9	−8.81	−12.64	−30.03	5.21	13.70	8.93
10	−8.90	−12.78	−30.84	5.27	13.78	9.06
11	−8.05	−11.28	−26.21	5.15	13.63	9.03
12	−8.05	−11.34	−26.27	5.17	13.64	9.04
13	−8.04	−11.29	−26.19	5.72	14.17	9.70
14	−8.29	−11.58	−26.58	5.10	13.58	8.99

Table 6-b. Electrostatic (G$_{el}$) and non-electrostatic (G$_{non-el}$) contributions to the solvation free energy for the conformers of Nod-B and Nod-A considered in Table 6-a.

3.6. Adducts with explicit water molecules

The study of adducts with explicit solvent molecules is particularly important when the solute molecule can form intermolecular H-bonds with the solvent molecule. Adducts with explicit water molecules were calculated for conformers expected to provide significant information. Fig.6. reports representative adducts of lower energy conformers of Nod-B and of the lowest energy conformer of Nod-A. The geometries of the adducts show features consistent with previous findings for APHLG [21], like the pentagonal shape of O atoms around the first IHB (counting the O atoms involved in the IHB and the O atoms of the three water molecules), which accounts for the hydrophobic nature of the region

B-5-d-w-9aq 39.201   B-3-d-r-c-8aq 36.698   B-4-s-w-a-13aq 40.696

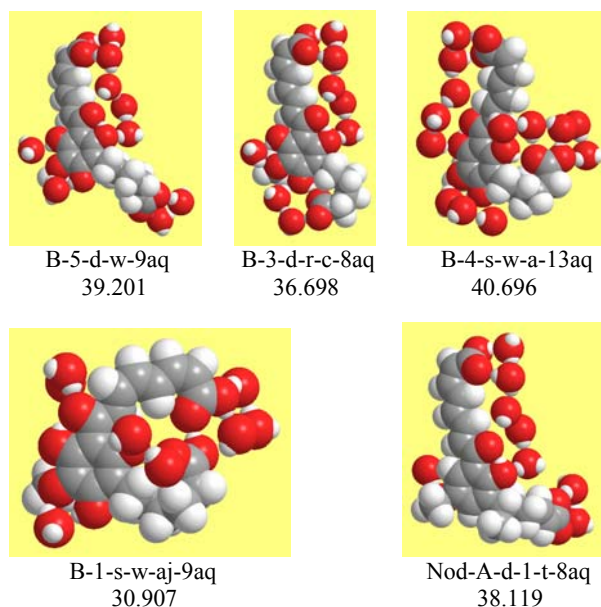B-1-s-w-aj-9aq 30.907   Nod-A-d-1-t-8aq 38.119

Fig.6. Representative adducts of Nod-B and Nod-A with explicit water molecules.
The adduct interaction energy (kcal/mol, corrected for BSSE) is reported under the name of each adduct.

conformer and IHB	H-bond parameters					
	O····H Å		O····O Å		OĤO	
	isol. mol.	add.	isol. mol.	add.	isol. mol.	add.
B-1-s-w-aj	1.747	1.792	2.575	2.613	143.0	142.6
H15···O32	1.953	1.975	2.850	2.880	157.1	158.3
H34···O24	1.882	1.808	2.823	2.761	166.9	170.2
H16···O11	2.111	2.325	2.654	2.741	115.0	105.7
B-3-d-r-c	1.684	1.663	2.531	2.518	145.3	146.0
H16···O32	1.991	1.877	2.888	2.786	157.3	158.6
H17···O11	2.057	2.310	2.620	2.732	116.5	106.0
B-4-s-w-a	1.728	1.612	2.559	2.479	143.4	147.1
H15···O32	2.002	2.092	2.888	2.958	155.0	150.9
H16···O11	2.116	2.300	2.656	2.727	114.7	106.6
B-5-d-w	1.699	1.678	2.540	2.528	144.6	145.5
H16···O11	2.221	2.431	2.720	2.781	112.0	101.4
H17···O11	2.163	2.228	2.683	2.693	113.4	108.9
A-d-1-t	1.762	1.717	2.589	2.560	143.2	144.7

Table 7. Comparison of the parameters of the IHB in the isolated molecule and in the adduct, for the adducts reported in fig.6. The parameters in the row with the name of the conformer correspond to the first IHB.

around the IHB. Table 7 shows the effect of the presence of the water molecules on the IHB of the central molecule, for the adducts reported in fig.6 and table 8 offers a comparison of the IHB linear parameters, including those from PCM results.

The comparison of B-5-d-w-9aq and B-1-s-w-aj-9aq (having the same number of water molecules) clearly highlights the importance of completely free COOH for the interaction between the central molecule and the water molecules, as shown both by

conformer and IHB	H-bond parameters					
	O····H Å			O····O Å		
	isol. mol.	aq PCM	add.	isol. mol.	aq PCM	add.
B-1-s-w-aj	1.747	1.780	1.792	2.575	2.598	2.613
H15···O32	1.953	1.954	1.975	2.850	2.850	2.880
H34···O24	1.882	1.885	1.808	2.823	2.830	2.761
H16···O11	2.111	2.214	2.325	2.654	2.741	2.741
B-3-d-r-c	1.684	1.731	1.663	2.531	2.568	2.518
H16···O32	1.991	1.952	1.877	2.888	2.848	2.786
H17···O11	2.057	2.174	2.310	2.620	2.732	2.732
B-5-d-w	1.699	1.739	1.678	2.540	2.573	2.528
H16···O11	2.221	2.256	2.431	2.720	2.714	2.781
H17···O11	2.163	2.285	2.228	2.683	2.734	2.693
A-d-1-t	1.762	1.758	1.717	2.589	2.588	2.560

Table 8. Comparison of the linear parameters of the IHB in the isolated molecule, in the PCM results with full reoptimization in water solution, and in the adduct with explicit water molecules, for selected cases. The parameters in the row with the name of the conformer correspond to the first IHB.

Fig.7. Adducts of the anions of B-5-d-w, considering the ionization of the COOH at the end of the acyl chain (a), of the COOH at the end of R′ (b) and of both COOH (ab).

anion	C23O24O25			C31O32O33		
	O24-aq	O25-aq	aq-aq	O32-aq	O33-aq	aq-aq
a	1.995 *	1.834	2.211			
b				1.942 *	1.810	2.263
ab	1.730 *	1.953	1.752	1.952	1.861 *	1.885

Table 9. Length of the intermolecular H-bonds in the anions of B-5-d-w (structures in fig.7). The asterisk denotes the H-bond with the water molecule that acts as donor in the aq-aq H-bond.

the interaction energy (reported under each adduct in fig.6) and by the adduct energies: although the energy of B-1-s-w-aj is considerably lower than that of B-5-d-w (table 2), the energy of B-1-s-w-aj-9aq is 4.911 greater than that of B-5-d-w-9aq. However, not all comparisons are as straightforward. The different accessibility, by water molecules, of the H-bond donor/acceptor centres in different conformers of the central molecule, the high number of possible arrangements of water molecules consequent to the high number of donor/acceptor centres (9 acceptor and 5 donors in Nod-B) and the tendency of water molecules to cluster together on optimization, make it difficult to obtain a set of adducts apt for comparisons providing information that can be related to the PCM results in water solution in an immediate way. It seems therefore appropriate to infer that, for middle-size molecules with many H-bond donor/acceptor centres, it would be convenient if PCM would take into account solute-solvent H-bonding directly [22], as the discrete/continuum combination (adducts with a limited number of water molecules, on which to perform PCM calculations) may become not viable or excessively demanding because of the size of the adducts and of their great geometry variability.

3.7. Adducts of the anions of Nod-B

The anions of carboxylic acids make strong H-bonds with water molecules. Adducts considering only the water molecules H-bonded to the COO⁻ groups were calculated (at HF/6-31+G(d,p) level) for the three anions of B-5-d-w (fig. 7), using the arrangement of water molecules that had proved optimal for the anion of the acid of phloroglucinol [23]. Table 9 reports the lengths of the intermolecular H-bonds.
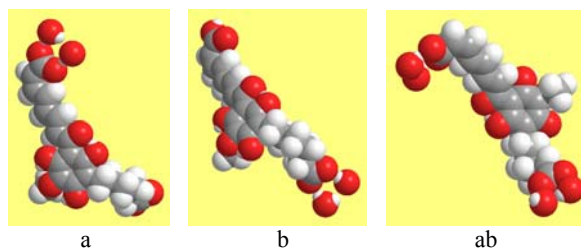
The C–O bond lengths in the COO⁻ groups become nearly equivalent in adducts a (1.231/C23-O24*, 1.242/C23-O25) and b (1.238/C31-O32*, 1.242/C31-O33), and less in ab (1.198/C23-O24, 1.308/C23-O25*, 1.199/C31-O32, 1.309/C31-O33*; the asterisk having the meaning explained under table 9). An anion-stabilizing role by the π conjugated system in the acyl chain is highlighted by the fact that the energy of b is 7.980 higher than the energy of a; correspondingly, the length of the first IHB is 1.682/a and 1.733/b (1.678/ab).

4 Conclusion

Comparisons of the results for Nod-B and Nod-A show determining influence of the hydroxybenzene moiety on conformational preferences and energies. The three equally-spaced OH of the phloroglucinol moiety in Nod-B enable several stabilizing features: the possibility of the H17···O14 option for the first IHB leaves the "right-hand" part available for other IHB, having a stabilizing effect *in vacuo* and in solvents with low/intermediate polarity; additional IHB can be formed between phenol OH and the ether O11. These results support the hypothesis that the phloroglucinol moiety has optimal stabilizing features among hydroxybenzenes derivatives.

The conformational preferences of Nod-B are consistent with those identified on model APHLG for what concerns the influence by specific features of the phloroglucinol moiety geometry, thus supporting the validity of considering the identified patterns as general for this class of compounds.

The conformers' relative energies from PCM

calculations in water solution appear to confirm that the first IHB does not break in water solution. Calculation of adducts with explicit water molecules confirm better solute-solvent interaction for conformers with only the first IHB; however, the search for an univocal answer about the permanence or breaking of IHB, other than the first, in water is complicated by the difficulty to obtain an adequately representative ensemble of adducts, what supports the potential conveniency of PCM models that could take into account solute-solvent H-bonds directly.

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