THE EFFECT OF ELECTROSTATIC PROPERTIES AND ABIBILITY TO FORM HYDROGEN-BONDS ON THE ACTIVITY OF BRASSINOSTEROID SIDE-CHAIN ANALOGS

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Dedicated to Dr Jan Fajkos on the occasion of his 75th birthday.

Molecular modeling studies on three brassinosteroids analogs having ester and amide function in the side chain have been performed. The activity of such compounds from the structural point of view is discussed in terms of electrostatic potential and ability to form hydrogen bonds.

Key words: Steroids; Brassinosteroids; Structure–activity relationship; Rice lamina inclination test; Electrostatic potential; MEP Map; GRID Map; H-Bonds; Molecular dynamics.

Brassinosteroids, potent plant growth regulators, have an exciting potential use in agriculture due to their capability of improving crop yield and quality as well as overcoming environmental stress and herbicidal injury, and controlling pathogenic diseases[1].

With the aim of looking for a more rigorous way to establish the structural requirements for a high brassinosteroid activity, a model based on brassinosteroid-receptor interaction has been described[2,3] which is useful in explaining the activity of different brassinosteroids from the structural point of view. Following this model, we have found that the electrostatic charges play an important role in explaining the activity and that the hydrogen-bonding could be one of the type of the interactions that could take place on binding. Based on the Grid methodology[4,5] over the set of brassinosteroids studied, the results obtained at present have allowed to provide defined information about the areas of the molecule responsible for binding and the ones eliciting activity[6]. The electronegative part of the side chain seems to be more important for exhibiting high activ-

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ity than the one of the A-ring diol. To asses this, some brassinosteroid analogs strongly differing in side chain should be studied.

In this communication, the activity of a set of brassinosteroids differing only in the side chain is discussed from two points of view: (i) the electrostatic charges of the functional groups on each side chain studied and (ii) the ability to form hydrogen-bonds. The brassinosteroids chosen have been previously synthesized by Kohout *et al.*[7] having ester (compound **1**) or amide (compounds **2** and **3**) function in the side chain but 2α and 3α hydroxy groups in the A ring and a 7-oxa-lactone grouping at the B ring (Figs 1 and 2).

The methodology of the study of the effect of electrostatic properties and ability to form hydrogen-bonds on the activity of such compounds consists of the following steps.

Activity Evaluation

Activity evaluation of the compounds has to be performed in the same bioassay. Thus, in a modified rice lamina inclination test[8], based on the procedure described by Takeno and Pharis[9], compounds **1**–**3** showed marginal activity, at least at doses lower than 2 μg per plant. It should be mentioned that in this bioassay it is not possible to apply higher doses due to the lack of assimilation by the plants. In fact, these compounds have been proved to be active in the bean second internode bioassay[7]. These findings are another example of the contradictions found when brassinosteroids are evaluated in different bioassays. The differences observed in the response of these brassinosteroids among bioassays suggest that different brassinosteroid actions are measured in each bioassay. Therefore, the specificity and usefulness of such bioassays must be treated with caution.

Active Conformation Approach

Our model is based on the fact that brassinosteroid action takes place through a receptor/ligand complex which binds to nuclear or cytoplasmatic sites to regulate the expression of specific genes. Since brassinosteroids can adopt different conformations due to the presence of flexible points in their structures, the "active conformation" of a brassinosteroid is defined as the one which is able to bind to the receptor. In this active conformation, the atoms directly involved in binding with the brassinosteroid receptor ought to have the same spatial situation in all active molecules. On the basis of this idea, an active conformation approach was developed which allowed us to choose first the active conformation for brassinolide (**4**), which is taken as reference, and then for a large set of brassinosteroids[3,6,10]. This approach consists in conformational analysis followed by the selection of the active conformation out of all conformations found in the analysis.

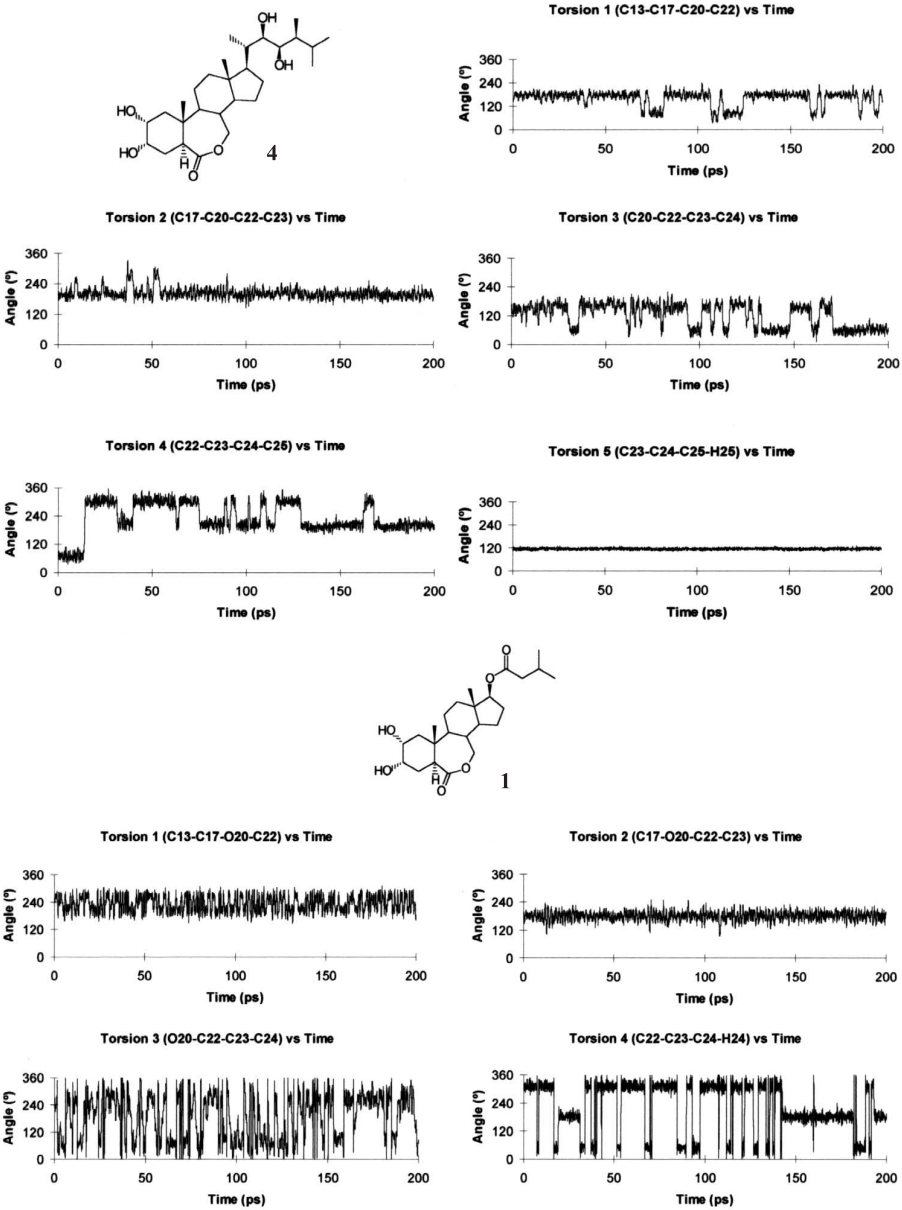


FIG. 1
Molecular dynamics under vacuum conditions and 900 K for brassinolide (**4**) and compound **1**

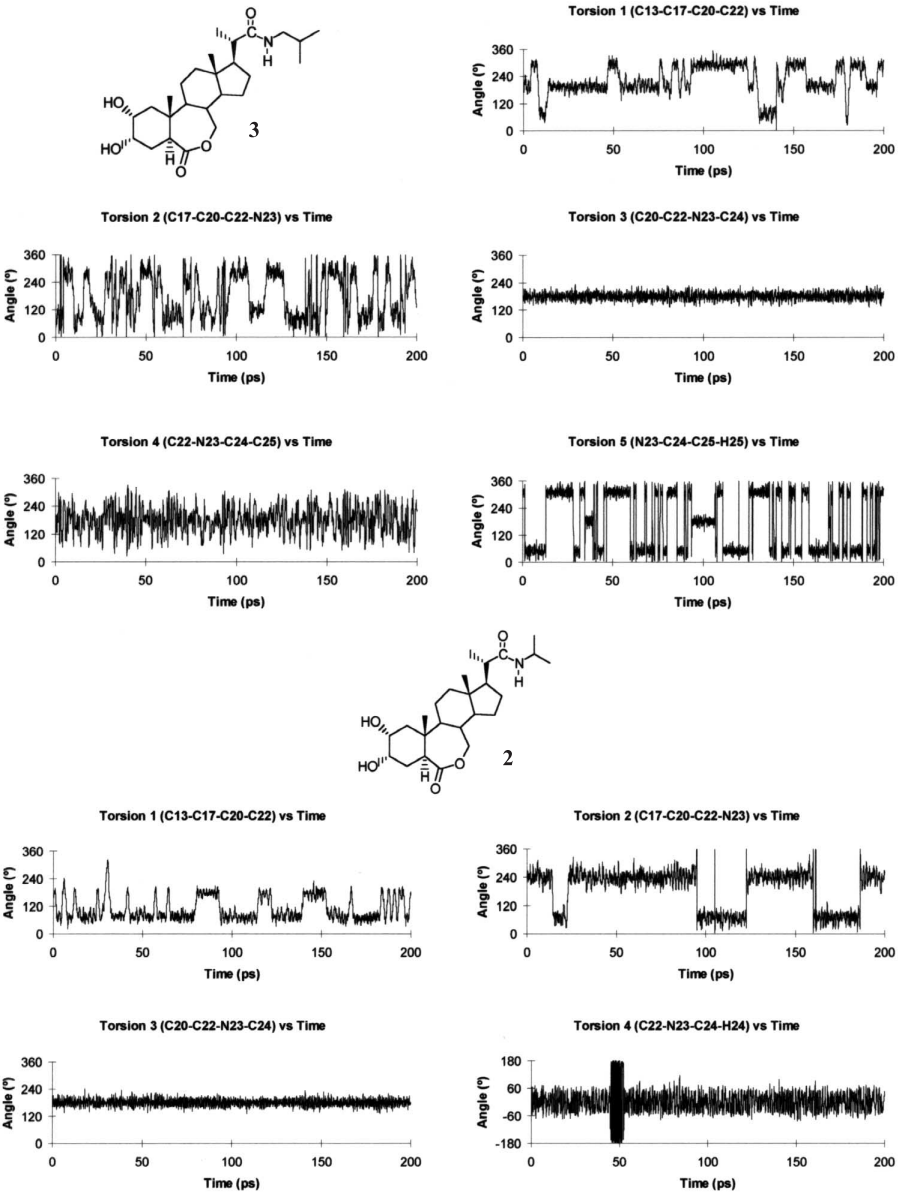


FIG. 2
Molecular dynamics under vacuum conditions and 900 K for compounds **2** and **3**

In this communication, due to the fact that the active conformation of brassinolide (**4**) is already known, a shortened methodology is followed to select the active conformation of the three compounds under study, **1**–**3**.

The side chain conformational analysis of **1**–**3** was performed by molecular dynamics simulation. Figures 1 and 2 show the different torsion angles obtained for each compound compared with those obtained for brassinolide (**4**). One can observe the high flexibility of all of these side chains compared with brassinolide (**4**), which means a large number of possible conformations that can adopted each of these compounds in a range of 5 kcal/mol.

To select the active conformation of each compound (which means the most similar to that of brassinolide (**4**)) out of all conformations found in the conformational analysis, a simultaneous optimization of similarity indexes and energy was performed with the ASP program[11]. Figure 3 shows the overlap of the active conformation found for compounds of **1**–**3**, and of that for brassinolide (**4**).

As one can see, there is a good overlap of the active conformations for all the compounds although they have different structural features in the side chains. The spatial situation of the functional groups present in the A and B rings of **1**–**3** are close to that in brassinolide (**4**). With respect to the functionalities of the side chain, the carbonyl groups of **1**–**3** are located near the 22-OH of brassinolide (**4**), whereas the oxygen of **1** and the amines of **2** and **3** differ a little more from the spatial situation of the 23-OH of brassinolide (**4**).

Molecular Electrostatic Potential Calculation

Having been determined by means of our preliminary QSAR (ref.[10]) that the atomic charges play an important role in describing the activity, the alignment of the structures

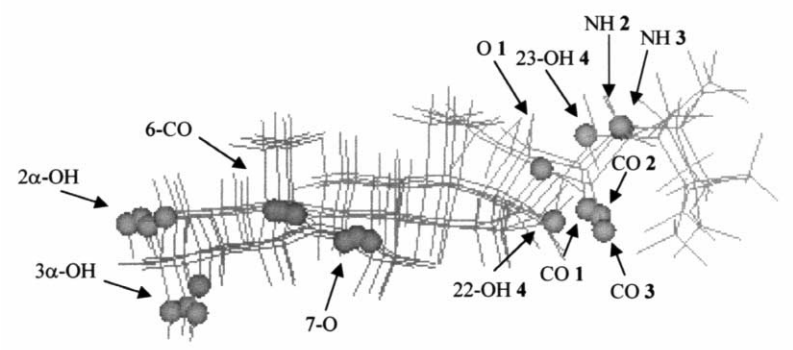


FIG. 3
Overlap of the active conformation for brassinolide (**4**) and compounds **1–3**

was performed by superimposing the molecular electrostatic potential (MEP) map of brassinolide (**4**) on that of compounds **1**–**3**.

Figure 4a shows the MEP map at –30 kcal/mol for brassinolide (**4**) in red and Fig. 4b the overlap its with those of the rest of compounds (in grey).

Two similarity indexes were calculated using the ASP program[11]: the electrostatic Carbó similarity index[12] (CI) and a shape similarity index (SI). The values obtained are indicated in Fig. 4. The high SI values obtained for these three compounds indicate the high structural similarity with brassinolide (**4**) although having their different side chains. On the other hand, the CI values for these compounds (0.72–0.78) show the some range as those calculated for some active natural and synthetic brassinosteroid analogs[3,6]. An example is found in Fig. 5 where (22*S*,23*S*)-24-epibrassinolide (**7**) and (22*S*,23*S*)-28-homobrassinolide (**8**) have CI values of 0.72 and 0.75, respectively. This indicates their great similarity in the electrostatic charge distribution, although **1**–**3** do not elicit activity in the rice lamina inclination test. Therefore, the structural modifications among them are not sufficient to produce a change in the global electrostatic similarity index. However, and in contrast with the results obtained for analogs differing in A and B ring[3,6], their activity does not correlate with CI values.

Feasibility of H-Bonding with the Receptor

A better explanation of the lack of activity of such compounds is found considering the possibility of hydrogen-bonding interactions with the receptor, which was specifically analyzed in our previous work over a set of brassinosteroids; the results were useful in explaining the activity of some of them[3,6].

The GRID methodology[4,5] has been used to calculate the interaction energy between a brassinosteroid and different probes which simulate different types of interactions. Among the probes tested, water has been chosen owing to its capability of acting both as acceptor and donor of hydrogen-bonds, N_3^+ (nitrogen atom of a protonated sp^3 amine type) as donor, and O:: (oxygen atom of a corbonyl group type) as acceptor.

For a better understanding, the results obtained with the three compounds under study, **1**–**3**. Figure 5 shows the GRID maps using water as a probe at a cut-off of –3 kcal/mol of the side chain overlap between brassinolide (**4**) (in red) and four brassinosteroids differing only in the side chain (in yellow): 28-homobrassinolide (**5**), 24-epibrassinolide (**6**) and their corresponding (22*S*,23*S*)-diastereoisomers **7** and **8**. The area in red correspond to the zone with higher probability of H-bonding for brassinolide (**4**) and the area in yellow correspond to the zone with higher probability of H-bonding for the rest of compounds. For compounds **5**–**8** a good qualitative correlation between the activity and the overlapped zone with higher probability of H-bonding between brassinolide (**4**) and each compound **5**–**8** is observed. The closer the zone in yellow for **5**–**8** is to that of brassinolide (**4**) (in red) the higher is their activity. Moreover, the zone corresponding with the 23-OH group (at the top of the picture) seems to be more im-

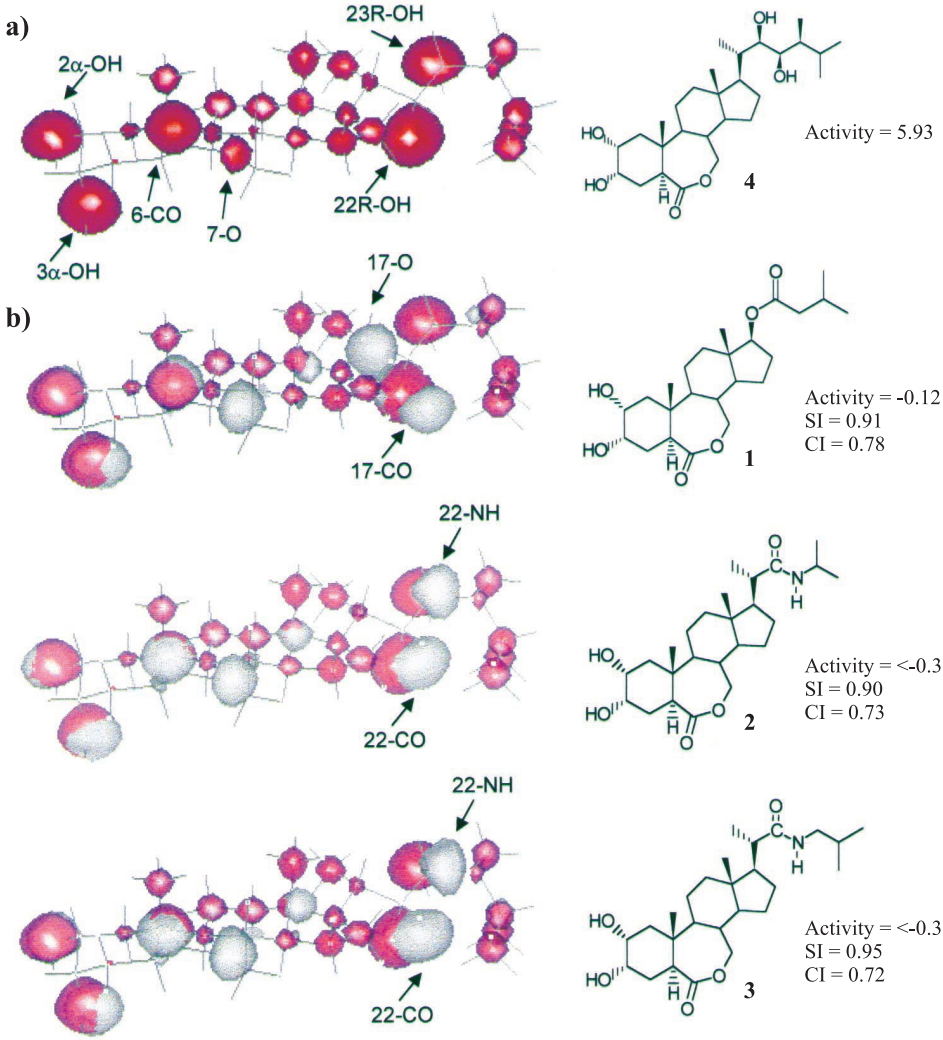
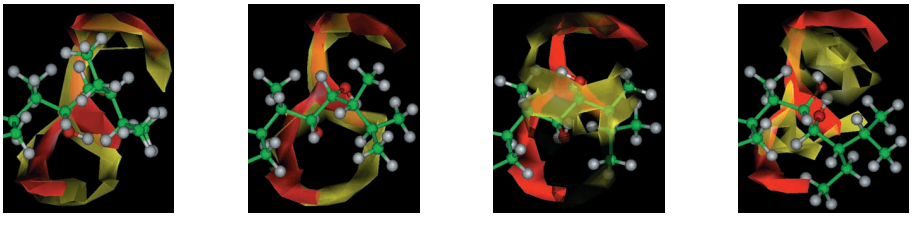
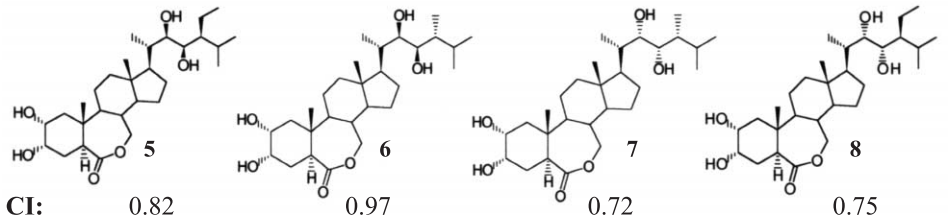


FIG. 4

A MEP map for brassinolide (**4**). b MEP maps overlapping between brassinolide (**4**) (in red) and **1**–**3** (in grey). Activity ($-\log(\text{dose})_{45°}$) in rice lamina inclination test, shape similarity index (SI) values, and electrostatic Carbó similarity index (CI) values

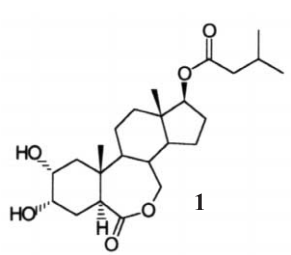


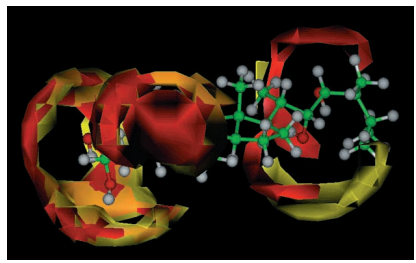


	5	6	7	8
CI:	0.82	0.97	0.72	0.75
Activity:	3.29	2.45	2.08	1.78

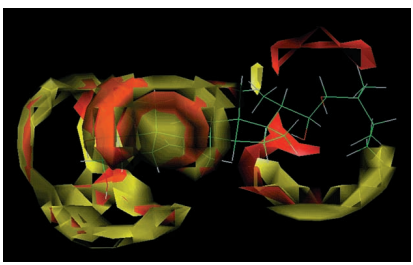
FIG. 5

Side chain BRs analogs. GRID maps overlap for water probe at –3 kcal/mol between brassinolide (**4**) (in red) and **5**–**8** (in yellow). Activity ($-\log(\text{dose})_{45°}$) in rice lamina inclination test, and electrostatic Carbó similarity index (CI) values

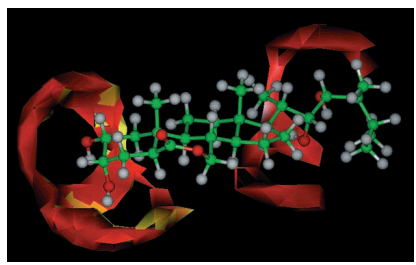




H_2O



N_3^+



O::

FIG. 6

GRID maps overlap for H_2O, N_3^+, and O:: probes at –3 kcal/molbetween brassinolide (**4**) and **1** (in yellow)

portant for binding with the receptor than the zone corresponding with the 22-OH group (bottom of the picture). As one can see, the difference between **4** and **5** is a shift to the right in the area corresponding to the 22-OH group, in agreement with the decrease in activity from 5.93 for **4** to 3.29 for **5**. This area is further shifted in the case of **6** and the activity falls again (2.45). For **7**, the activity of which is 2.08, the area corresponding to the 22-OH is located in another plane, and only a overlap is observed between the areas corresponding to the 23-OH. Finally, for **8**, with an activity of 1.78, for which no overlap is observed between the two hydroxy groups of the side chain but the area corresponding to the high probability of H-bonding for the 23-OH of **4** is not so far from that of **8**. Hence it seems that the area with the highest probability of H-bonding must be located in a specific zone to express activity.

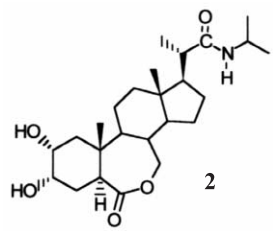
Similar methodology is used for compounds **1**–**3** but with different probes. Thus, Fig. 6 and 7 show the GRID map at a cut-off of –3 kcal/mol for brassinolide (**4**) (in red) overlapped with each one of the other compounds, **1**–**3** (in yellow) using (i) water, (ii) N_3^+, (iii) O:: as probes.

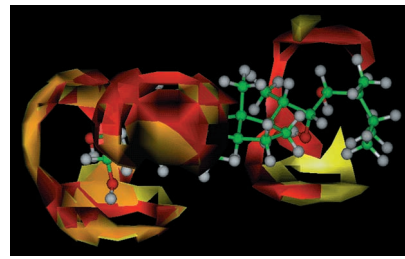
By comparing these three compounds with brassinolide (**4**), one can observe that the larger differences is found in the side chain, in accordance with their structural features. While in some cases the sites corresponding to a functionality on the skeleton of **4** lays in front of those of the other compounds **1**–**3**, the red area being over the yellow one; in the cases of compounds **1**–**3** which lays in front of **4**, the yellow area being over the red one.

Due to the fact that the ester carbonyl in **1** is located near the 22-OH group of **4**, no interaction is observed in the area close to the 23-OH group of **4** in any of the probes tested; only an interaction near the 22-OH group is observed when H_2O and N_3^+ are used as probes, being in the last one highly shifted to the right with respect to that in brassinolide (**4**).

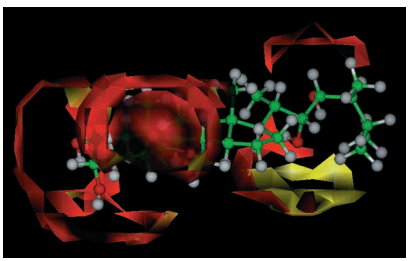
For amides **2** and **3**, with carbonyls located near the 22-OH group and the amino group near the 23-OH group only a very small interaction is observed in the area corresponding to the 23-OH when H_2O and O:: are used as probes. For the 22-OH group the same results as for compound **1** are observed.

On the basis of the results obtained over the set of side chain brassinosteroid analogs, we can conclude that the activity exhibited by brassinosteroids can be explained by considering the putative H-bonding interactions in the brassinosteroid-receptor complex. Due to the lack of activity for compounds **1**–**3**, the area located near the 23-OH group with the highest probability of H-bonding seems to be essential for exhibiting activity when it is located in a specific zone. Nevertheless, at present we are not able to discriminate if the 23-OH acts as H-bond donor or acceptor since brassinolide has shown strong interactions in this area whatever it was the probe used. Moreover the 22-OH group was responsible for activity, and taking into account that the interaction in this zone for compounds **1**–**3** is only observed when H_2O or N_3^+ is used as probe, this interaction ought to be H-bond donor.

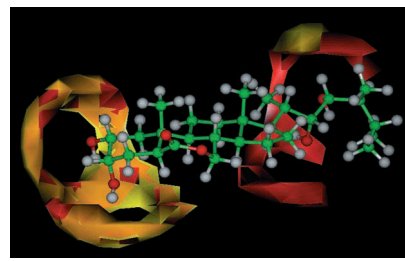




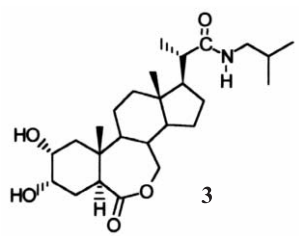
H_2O

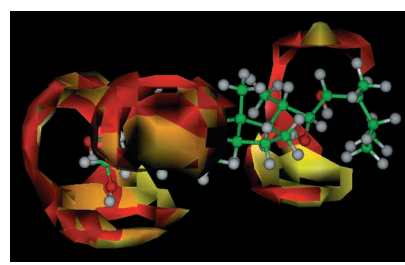


N_3^+



O::





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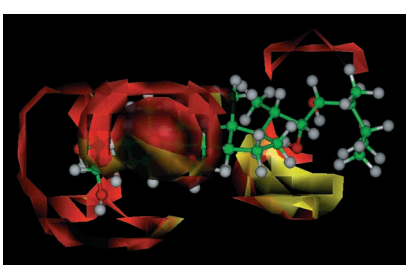
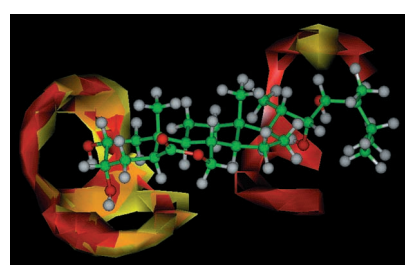


N_3^+



O::

FIG. 7

GRID maps overlap for H_2O, N_3^+, and O:: probes at –3 kcal/mol between brassinolide (**4**) (in red) and **2**–**3** (in yellow)

Further studies over brassinosteroids having other functionalities in the side chain need to be performed to provide more information about these findings.

EXPERIMENTAL

Rice Lamina Inclination Test

Seeds of Bahia rice cultivar were soaked in water and incubated in a growth chamber under a 16 h light/8 h dark photoperiode at 30 °C for two days. Germinated seeds were planted on the surface of 0.5% aqueous agar medium and incubated under the above conditions for four days. Selected seedlings were treated with brassinosteroid test solutions (95% ethanol) by applying them with a microsyringe (0.5 μl) to the second lamina joint of the plant sheath. Treated and untreated (control) seedlings were returned to the growth chamber at 30 °C in the dark for two days. Then the interior angle between the leaf lamina of the second leaf and its leaf sheath was measured and statistical parameters calculate.

Molecular Dynamics Simulation

Molecular dynamics simulation was performed using the CVFF force-field included in the molecular modeling software package Discover[13]. The conditions for this analysis were the following: vacuum simulation, time step (2 fs), duration of simulation (200 ps), integration algorithm (Verlet), ensemble (NVT) with a temperature control (velocity scale at 300 K).

Molecular Electrostatic Potential Calculation

The molecular similarity program[11] ASP was used to calculate the electrostatic potential for each compound and to superimpose them. The electrostatic potential was calculated using the gaussian function option with three terms to fit the distance inverse dependence. The electrostatic Carbó similarity index[12] (CI) and a shape similarity index were also calculated using the ASP program.

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