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Antiradical activity of isoquinoline and indole alkaloids

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Abstract

The antiradical activity of four alkaloids -ibogaine, voacanginol, boldine and berberine- were studied. The relationship between their antiradical activities and structural aspects was investigated. The antiradical activity was tested in ethanolic solution as the ability to scavenge free 1,1'-diphenyl-2-picrylhydrazyl radicals (DPPH). The lowest energy molecular conformations and electrostatic potential of the alkaloids were calculated using HyperChem molecular modeling software. Three compounds ibogaine, voacanginol and boldine bear abstractable hydrogen on their structures; the main difference between them is the presence of phenolic hydrogen in boldine and labile hydrogens linked to nitrogen atoms in ibogaine and voacanginol, two closely related alkaloids. The alkaloid tested boldine showed similar activity to voacanginol and ibogaine, whereas berberine, lacking an abstractable hydrogen in its structure, showed the lowest antiradical activity.

Key words: berberine, boldine, ibogaine, voacanginol, DPPH.

Actividad antiradical de alcaloides indólicos e isoquinolínicos

Resumen

Se realizó el estudio de la actividad antiradical de cuatro alcaloides: ibogaina, voacanginol, boldina y berberina. Se investigó la relación estructura-actividad antiradical para los alcaloides estudiados. La actividad antiradical fue evaluada como la habilidad para neutralizar el radical 1,1´-difenil-2-picrilhidrazilo (DPPH). Se determinaron las conformaciones de menor energía y el potencial electrostático asociado a los compuestos evaluados utilizando el programa HyperChem. Ibogaina, voacanginol y boldina poseen hidrógenos lábiles en sus estructuras. Ibogaina y voacanginol, dos alcaloides relacionados, poseen hidrógenos unidos a un átomo de nitrógeno en un anillo indólico, mientras que ibogaina, posee hidrógenos fenólicos. Boldina muestra una actividad antiradical similar a la obtenida para ibogaina y voacanginol. Berberina, que no posee hidrógenos lábiles en su estructura mostró la menor actividad antiradical.

Palabras clave: berberina, boldina, ibogaina, voacanginol, DPPH.

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Introduction

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foods. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources (1-6). Free radicals and other related species cause the oxidation of biomolecules (e.g., protein, amino acids, lipids and DNA) and it is now recognized that free radicals contribute to more than 60 diseases such as cancer, Parkinson, Alzheimer, diabetes, and aging (7-9).

Since ancient times, spices used to improve flavors in different types of food are well known for their antioxidant capacities (3, 5). In recent decades, the essential oils and various extracts of plants have been of great interest due to their antioxidative capacity (1, 2, 6, 10, 11, 12).

The study of antiradical compounds has been applied to the development of effective biological antioxidants. Thus, the discovery of compounds of natural origin that possess an efficient trapping mechanism, that can be used as antioxidants, is an objective of research in different areas of science.

Many studies have been published concerning the trapping of Radical Oxygen Species by colored radicals. In particular, the diphenylpicrylhydrazyl (DPPH) is widely used for quickly assessing the ability of antioxidants to transfer labile H atoms to radicals (3-5, 12). Previous studies suggested that the reaction between DPPH and phenolic compounds is more like an electron transfer reaction rather than a hydrogen atom transfer (13). In the DPPH test, the antioxidative capacity is characterized by the bleaching of the colored solution of DPPH with the sample followed by UV-vis spectrometry and quantitatively by the EC_{50} value (concentration necessary to reduce 50% of DPPH) or their stoichiometry. Isoquinoline alkaloids are a numerous group of secondary metabolites including among others, oxoaporphine, protoberberine, aporfine, and bisbenzylisoquinoline alkaloids. Protoberberine and aporphine alkaloids represent almost half the isolated alkaloids in plants of the genera belonging to *Guatteria*, *Berberis* and *Coptis*. The aqueous and alcoholic extracts of plants containing isoquinoline alkaloids are active against bacteria and fungi. In addition, some studies showed that protoberberine alkaloids and related compounds possess antiphotooxidative and antitumor activities (14, 15).

Berberine (**I**) is an organic cation isolated from numerous plants of the genera *Berberis*, *Mahonia*, and *Coptis*, and has shown to exert a broad spectrum of antiradical, antioxidant and antitumoral activities (15-17). Boldine (**II**), an aporphine alkaloid, isolated from plants belonging to the *Boldus* genera has been the object of numerous biological studies (18-21).

Ibogaine (III) and voacanginol (IV) have been isolated from several species of *Iboga*, *Voacanga* and *Tabernaemontana* genera, and iboga alkaloids have shown antiulcerogenic, antileishmanial and antiadditive activities (22-28).

A major difference between these alkaloids is the presence of phenolic hydrogen in boldine (II) and labile hydrogens linked to nitrogen atoms in ibogaine (III) and voacanginol (IV).

The aim of this study was to contribute to the understanding of antiradical activity of the alkaloids **I**, **II**, **III**, **IV** (figure 1) with respect to their structural features and physico-chemical properties. The antiradical activity was tested in ethanolic solution as the ability to scavenge free 1, 1'-diphenyl-2-picrylhydrazyl radical (DPPH). The lowest energy molecular conformations and the highest occupied molecular orbital (HOMO) of the alkaloids were calculated using HyperChem molecular

modeling software. The results demonstrate a feasible correlation to the structure-activity relationships in the compounds (I-IV) studied.

Materials and methods

Chemicals

Berberine hydrochloride and 1,1'-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Boldine hydrochloride was purchased from Fluka Chemical Co. (Buchs, Switzerland).The pure iboga alkaloids ibogaine (**III**) and voacanginol (**IV**) were kindly provided by Dra. Deanna Marcano (Universidad Central de Venezuela) and identified by NMR spectroscopy. Copies of the original spectra are obtainable from the author of correspondence. All other chemicals were of highest commercial grade and used without further purification.

Software

Molecular mechanics MM+ packages provided by Hyperchem were used.

DPPH radical scavenging assay

Samples (I-IV), were added to DPPH solution (prepared fresh daily) in methanol, to give the final concentration of 10, 30 and 60 μM for the antiradical and 60 μM for the DPPH. The absorbance decrease of the reaction mixture was continuously recorded at λ_{max}=515 nm using an OceanOptics spectrometer. The control consisted of the same quantities of 99% ethanol and DPPH radical solution. The DPPH radical scavenging activities of the test compounds were expressed as the % remaining DPPH radicals at each time point. All samples were carried out in triplicate. The total antioxidant capacity (TAC) of each tested compound was quantified as the relative absorbance of the solution measured at 515 nm, 45 min after the reaction started. The disproportion is the relative quantity of alkaloids which react with DPPH calculated as the difference on the absorbance at time infinite vs absorbance a time zero. The reaction rate constant k was calculated by fitting the experimental values to a first order decay equation.

Results and discussions

In order to assess the radical scavenging potential of the compounds tested (figure 1), the reactivity toward the stable free radical DPPH was measured as the lack of absorbance at 515 nm of an ethanol solution of DPPH containing the compound tested. The kinetic decay absorption curves for different concentrations of the alkaloids with DPPH solutions are shown in figure 2. In all the cases the plots shows the evidence of the existence of two time regimes, in the first 500 s, more than 80% of the reaction occurs. To achieve the end of reaction it is necessary to measure continuously until 3000 s. The reaction rate k in the first step is related to the facility of the test compound to interact with DPPH, while the amplitude of the curve is related to the efficiency of the interaction. The short time constant varies for each compound tested, while the long time constants are similar and independent of the sample. These results prove that the kinetic constants calculated at the short time period are related to the efficiency of the reaction. Kinetics constants calculated at the long time period correspond to the diffusion constant of the molecules in the solvent; this result is in accord that observed in previous studies (2).

In the first step of the reaction, ibogaine (**III**), voacanginol (**IV**) and boldine (**II**) showed an increasing value in reaction rate constants. In the case of berberine (**I**), the decay constant is the smallest, reflecting the low antioxidative property of this compound, in agreement with previous studies (2).

The DPPH has a free radical which can be stabilized throughout the aromatic rings of the core structure. Due to this property

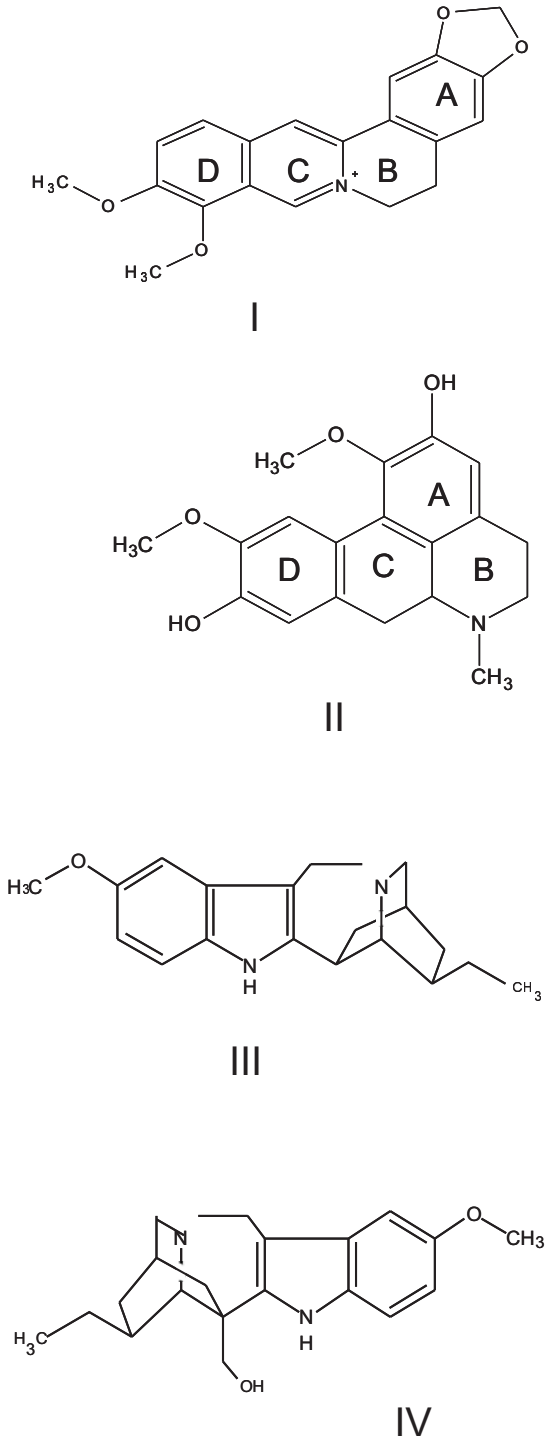

Figure 1. Chemical structure of the alkaloids tested: berberine (**I**), boldine (**II**), iboagaine (**III**) and voacanginol (**IV**).

this compound is useful to test the free radical scavenging potential of compounds in solution with it. There are two principal ways to achieve the free radical stabilization between a test molecule and DPPH. The first is by formation of a covalent bond with the nitrogen 2 of the DPPH and the second is by charge complex formation. In the former cases it is necessary that the test molecule can delocalize the generated free radical in the reaction. In the latter, the interaction occurs by the sharing of charge density.

Figure 3 shows the disproportion as a function of sample concentration for the different alkaloid solutions. For berberine (**I**), we observe that only a small fraction of the DPPH is consumed, and this value is almost constant in the range of concentrations studied. This polycyclic structure does not contain phenolic groups or labile hydrogen atoms. The interaction of DPPH with this molecule should be through a charge transference complex formed between DPPH and the methylenedioxy group. In previous work we have found that the oxygen lone pair electron orbitals of the methylenedioxy group on berberine (**I**) are perpendicular to the aromatic ring A while vicinal methoxy groups are held out of the plane of the aromatic ring to avoid steric compression (15). This disposition can be responsible of the charge transfer complex mentioned above. The 3D map of the highest occupied molecular orbital (HOMO) for berberine (**I**), revealed a concentrated zone in the vicinity of the methylendioxy groups (figure 4a).

Boldine (**II**) possesses two phenolic groups that have labile hydrogen atoms and the resulting negative charges on hydrogen abstraction in each case can be stabilized by the adjacent aromatic rings. The HOMO map of this molecule supports the idea of hydrogen radical abstraction from both the phenolic hydrogens, since the 3D HOMO representation shows a high concentration in almost all the molecule (figure 4b). The direct interaction of DPPH with the phenolic groups shows a high reaction rate. The dis-

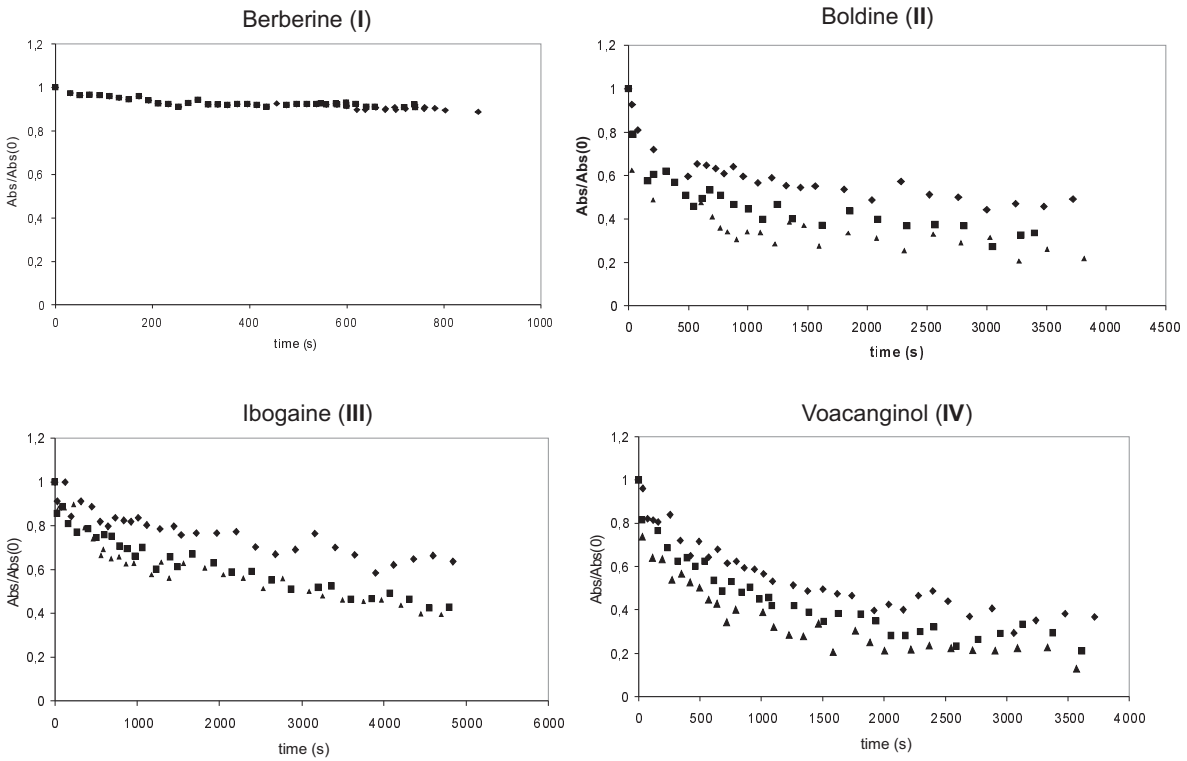

Figure 2. Kinetics decay absorption for different concentrations of the alkaloids with an ethanolic solution of 1,1´-diphenyl-2-picrylhidrazyl (DPPH) radical (60 µM) in presence of the antioxidants observed at λ_{max}= 515 nm. ♦ 10 µM, ■ 30 µM ▲ 60 µM.

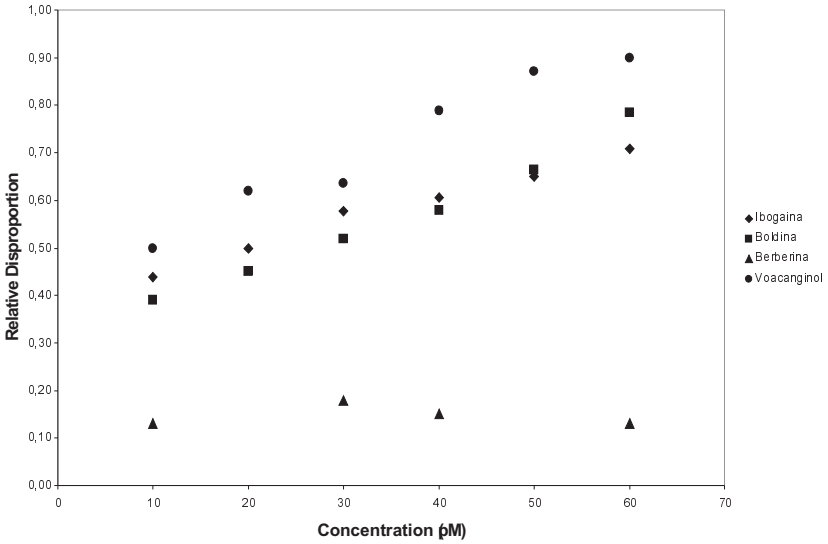

Figure 3. Disproportion vs concentration of tested alkaloids with an ethanolic solution of 1,1´-diphenyl--2-picrylhidrazyl (DPPH) radical (60 µM) in presence of the antioxidants observed at λ_{max}= 515 nm. ▲ berberine, ● boldine, ♦ ibogaine, ■ voacanginol.

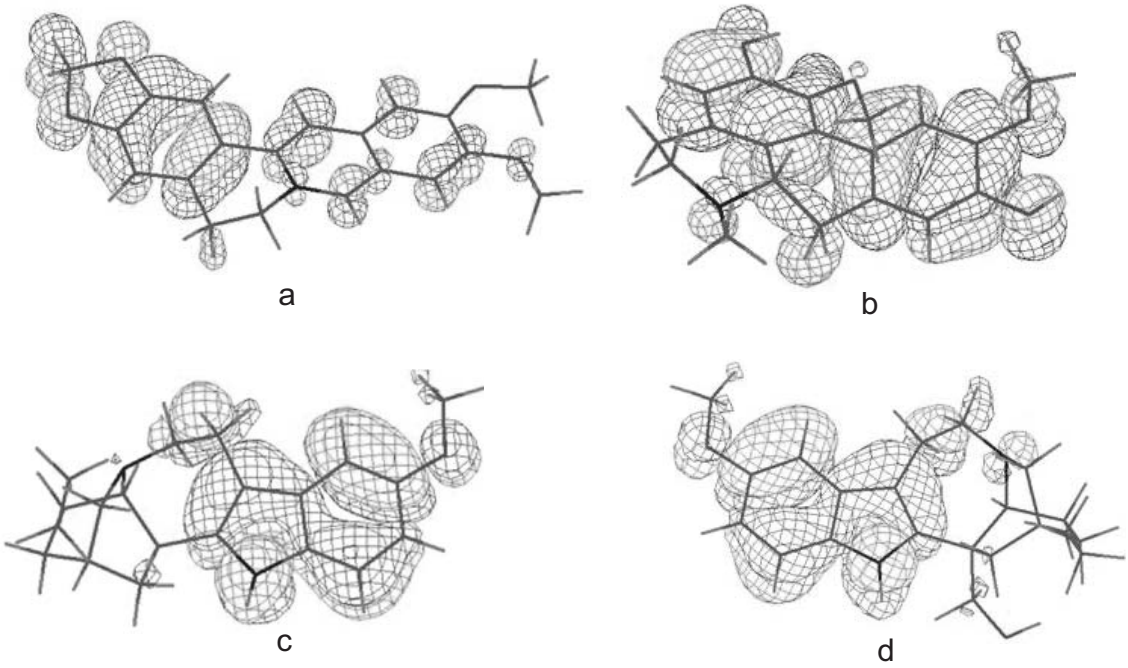

Figure 4. 3D maps for the highest occupied molecular orbital (HOMO) density of the more stable conformers of the compounds: **I** (a), **II** (b), **III** (c) and **IV** (d).

proportion for this molecule (figure 3, circles) is the highest of the evaluated group and consequently reflects the high antioxidative activity. Previous studies have been show that phenolic groups on the aromatic rings A and D strongly influence the antiradical activity (2).

In the case of Ibogaine (**III**) and voacanginol (**IV**), indole alkaloids with similar features to an antioxidant standard stobadine (2), the interaction of the DPPH is through the hydrogen attached to the nitrogen present in the indolic nucleus. The HOMO maps show the highest concentration in the surroundings of these centers for the two alkaloids (figure 4c and 4d). According to our results, boldine, bearing unsubstituted –OH groups proved to be able to scavenge free stable DPPH radical, similar to that shown by ibogaine (III) and voacanginol (IV). In contrast, the interaction of ibogaine and voacanginol with DPPH occurs through an N-H labile bond.

Conclusions

We found that boldine, ibogine and voacanginol could act as potent scavengers of DPPH radicals. The presence of free hydrogen labile bond on the skeleton was shown to be essential for good intrinsic antioxidant activity.

Acknowledgements

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