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Proceeding Paper

Getting into Structure-Activity Relationships of Ecdysteroids for Plant Protection Strategies against Insect Pests †

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Abstract: The transformations of insects and typical invertebrates during the larval state (metamorphosis) in their life cycle is an example of the chemical signal systems in cell-internal control mechanisms. The ecdysone, following the classic steroids' action mechanism, controls the morphogenesis and metamorphosis and its chemical reactivity, as a quantitative term, depends on optimum geometric, electronic and chemical properties. Ecdysteroid activity of several naturally occurring and synthetic steroidal derivatives have been studied previously, and notable results have been achieved. This paper intends to achieve the following: (1) quantitatively determine which key structural points of the ecdysone molecule and analogues trigger the bio-functional action, starting from the structure–activity relationship and the analysis of electronic properties, using a very simple protocol; (2) propitiate a mathematical-statistic tool that allows the discrimination of the most active molecules from the less active ones, optimizing the synthesis of steroidal analogs with defined ecdysteroid action, useful for applying in field conditions for controlling insect pests which affect crop yields.

Keywords: ecdysteroids; structure-activity relationship; synthesis; plant protection; steroids

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1. Introduction

The evolution of organisms is a result of a complex and changing interactions (*geno* and *pheno*) with the environment at both a global, planetary scale and population level. The cell, a biological unit formed by "powerful and extraordinary efficient chemical machinery" used to provide it with energy, self-maintenance, exchange of materials with the exterior and an amazing chemical signal system for communication (both intra and inter), is not completely autonomous in its functions, even in unicellular organisms. The multicellularity concept is necessary to guarantee a successful cell operation, which allows a more efficient specialization of the plasma in continuously changing environmental conditions. During the development process, three fundamental points define, evolutionarily, the success of it: (1) the cell-internal control mechanisms; (2) external control of the cell; and (3) links between the environment and genes. This "social control" is a guarantee of the homogenous development of an entire organism [1,2].

The transformations during the development of insects and typical invertebrates during the larval state (metamorphosis) in their life cycle is an example of the chemical signal system, as mentioned above in point 1. The ecdysone, the so-called "moulting and metamorphosis" hormone, following the classic steroids action mechanism [3], is produced by the prothoracic glands after activation induced by neuropeptides [4]. This compound (and its derivatives) controls the morphogenesis and metamorphosis jointly with juvenile hormones [5].

In biological systems, where target–compound interactions define the physiological functionality, chemical reactivity is a quantitative term, and it depends on optimum geometric and physicochemical properties. The ecdysteroid activity of several (>300) naturally occurring and synthetic steroidal derivatives, starting from ecdysone, have been studied over the past 20 years [6]. However, the structural elements required for the hormonal activity are not clear. Therefore, this paper intends to achieve the following: (1) quantitatively determine which key structural points of the ecdysone molecule and analogues trigger the bio-functional action, starting from the structure–activity relationship and the analysis of electronic properties, using a very simple protocol; (2) propitiate a mathematical-statistic tool that allows the discrimination of the most active molecules from the less active ones, optimizing the synthesis of steroidal analogs with defined ecdysteroid action, useful for application in field conditions for controlling insect pests which affect crop yields.

2. Materials and Methods

Simple and quick research into computational chemistry was necessary for understanding geometric and quantum descriptors. The study of the ecdysteroid molecules was carried out starting from elaborated designs in HYPERCHEM software, beginning with a geometry optimization with molecular mechanics (MM3). The quantum calculations were made at a semi-empirical level using PM3 formalism with MOPAC 6 program. The main conceptual points considered were: optimization of geometric structure of ecdysone and; evaluating of each functional group and its molecular significance on optimized ecdysone. HYPERCHEM 5.02 was used for illustrating the molecular orbitals as well, with ZINDO/S option.

After that, the following molecular properties were calculated: ionization potential; energy of frontier orbitals (HOMO and LUMO) and contiguous orbitals from HOMO–1 to −5, and from LUMO +1 to 3; atoms providing a higher contribution to these molecular orbitals; oxygen atoms contributing to the HOMO–3 and HOMO–4 orbitals; spatial distribution of HOMO, LUMO and HOMO-3 orbitals; distances between intermolecular atoms, valence and torsion angles (τ) among all of the ring's atoms in the molecules; charge of each atom. At the end of the calculus, 167 variables per molecule were available [7].

Statistical analysis on the analogues and ecdysone (Figure 1), including mean statistical characterization from the geometrical and quantum points of view, was done. Therefore, we aimed to discriminate the variables implicated in biological activity. The statistical analysis was carried out with descriptive and decisional techniques such as cluster analysis, discrimination analysis with Lambda of Wilks methods, and *t* test (everything was made with SPSS statistical package). Every computer calculus was done on IBM-PC Dual Pentium II, 750 MHz, 1 GB of RAM memory.

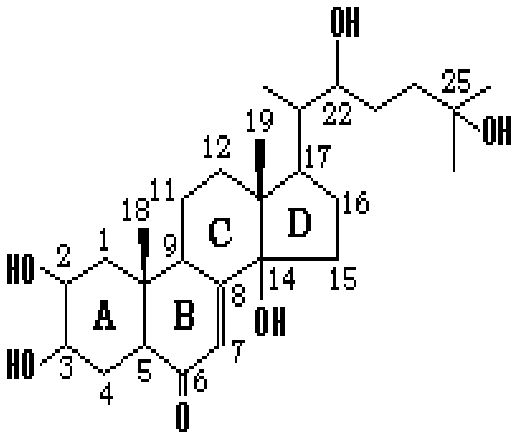

Figure 1. Ecdysone and structural core with atom numeration used.

3. Results and Discussion

The analysis of the presence/absence of OH groups and other functional groups was the first step. The existence of OH in position C22 (Figure 2) and the functional relationship of the OH in positions C5 and C14 are of little importance for making a discrimination between physiologically active or inactive molecules compared to ecdysone molecule. However, these variables do not guarantee a strong discrimination. It seems to be that the existence, or lack thereof, of functional groups (generally OH) do not contribute a total explanation of a functional dependence between the structural properties and the biological activity [8].

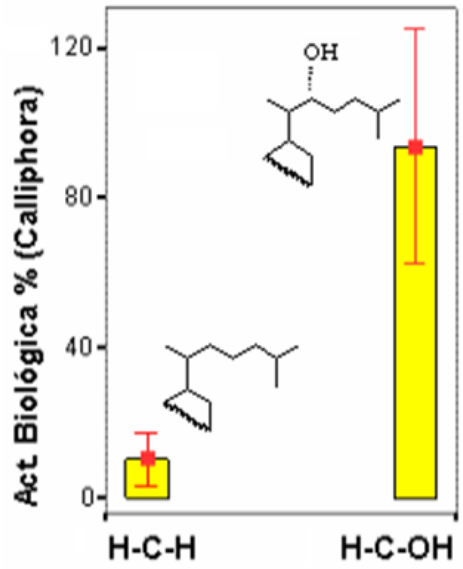

Figure 2. Biological activity with and without α OH in C22 (t = 0.04517).

Taking this in consideration, we can demonstrate which functional groups are the fundamental structural cause in the increment, or decreasing, in the biological activity of a given molecule, in comparison with ecdysone. However, it is obligatory to analyze where other properties that refer to the whole molecule are included for quantifying the relation between molecular structure and physiological function.

Hence, a rigorous chemical–biological functional analysis via statistical and mathematical methods was conducted.

Using discriminate analysis, structurally important variables for the functional dependence between ecdysteroids and their biological activity were determined:

* Distance O3–O22; O22–HOMO3;
* Ang 2,3,4; Ang 8,9,10;
* Distance C2–C3;
* O3 atom with major contribution to HOMO–3;
* Charge (q) at C16.

Later, a regression equation [F = 0.00461009; R^2 = 0.873467] was found in order to create an approximation of the free energy of ligand–receptor binding [ΔG^0_{bind}] [9,10], resulting in a general equation:

$$\text{Log (Y-0.5)} = -46.184 + 1.356\ (\text{O22-HOMO3}) + 0.483\ (\text{O3-HOMO3}) + 0.398\ (\text{Dist. O3-O22}) + 21.902\ (\text{Dist. C2-C3}) - 0.258\ (\text{Ang. 2,3,4}) + 2.229\ (\text{Ang. 8,9,10}) - 94.714\ (\text{q16})$$

If:

Dist. O3–O22 is x; Dist. C2–C3 is y; Ang. 2,3,4 is z; Ang. 8,9,10 is v and q 16 is w.

The equation becomes:

$$Y = 10^{-46.184+1.356\ (O22\ HOMO3)+0.483\ (O3\ HOMO3)+0.398x+21.902y-0.258z+0.229v-94.714w}$$

The derivatives analyzed are typical polyhydroxy compounds and are characterized by their capacity to generate H-bonds (3 Kcal/mol). Such bonds can, via stereochemical orientation of the OH groups, determine some functional properties as specific enzyme activities [11]. It is necessary to highlight that the use of this equation allows to recognize very precise and coincident properties, regions and atoms in these molecules under study and correlate with the properties of molecular systems based on ecdysone derivatives (Figure 3).

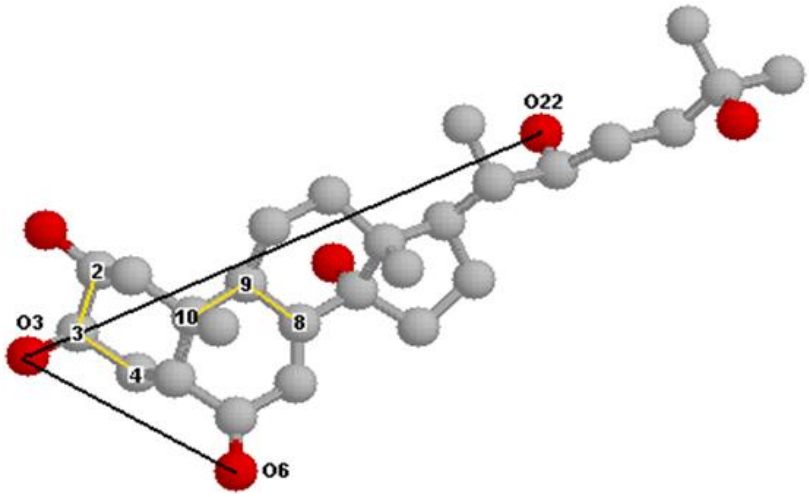

Figure 3. Spatial representation of the main molecular variables in ecdysteroids related to their biological activity. Interatomic distances (represented by black lines), angles (represented by yellow lines), the atoms O3 y O22 are key contributors to HOMO-3 orbital and the O6 is the major key contributor to HOMO.

From the results, the following is inferred for making a prediction of biological activity from the molecular structure: (1) some functional groups, and sub-structural units, are indispensable as part of the molecule, as well as their spatial distribution and electronic properties, for developing any interaction between target receptor and ecdysteroid compounds; (2) additionally, these selected functional groups should contribute to the properties of the whole molecule (e.g., the contribution of the atoms O3 and O22 to molecular orbital HOMO–3). Necessarily, the regularities of different kinds of reaction between the ligand and receptor should be taken into account.

In fact, there is agreement with [12] as well: oxygen atoms are important, however, from a spatial orientation point of view, and an interaction with the enzyme or receptor, not all of them. The ligand, a plane molecule of a steroid, will not need more than two bindings points, at molecular scale, with the receptor, minimizing the spent of energy in bonds.

If the hydroxyls (–OH) perform a function on electronic structure of the molecules [13], then we should not reduce the influence from these atoms to simple spatial disposition. Once the molecule has been bonded through the O22 and O3 positions, a distance restriction will be necessary to considerer.

Finally, using a very simple computational protocol, and taking into consideration the data reported, the process of an ecdysteroid molecule binding to a receptor develops via Side Chain binding/A, B-ring acting, similar to other steroids [14]. The results derived from this research, currently, are applied in the synthesis of polyhydroxy- and keto spiro-steroidal derivatives, starting from steroidal sapogenins, with quite remarkable ecdysteroid activity and applicability in eco-sustainable conditions, for controlling insect pests and vectors. The synthetic protocol and evaluation of ecdysteroid activity will be published soon.

Author Contributions: Conceptualization J.E.T.M. and J.C.S.; methodology, J.C.S. and J.E.T.M.; software, M.B.F.; validation J.C.S., J.E.T.M. and R.Q.; investigation and resources, J.E.T.M., N.E.S.; writing—original draft preparation, review and editing R.Q. and J.E.T.M.; visualization and supervision, M.B.F. and N.E.S.; project administration J.E.T.M. and J.C.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest. The Technical University of Esmeraldas, Ecuador, had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. Authors declare that the research done is for pedagogical purposes only, and for comprehension of significance of computational chemistry, and its implementation is intended as a tool for designing new molecular entities.

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