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Application of Hansch's Model to Guaianolide Ester Derivatives: A Quantitative Structure−Activity Relationship Study

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A quantitative structure−activity study to evaluate the effect of lipophilia/aqueous solubility on etiolated wheat coleoptiles elongation has been carried out with 34 guaianolides having different numbers of hydroxyl groups and ester side chains of variable length and structure: linear, branched, aromatic, and unsaturated. Compounds have been tested in a range of concentrations between 10 and 1000 μM. Data show a strong influence of lipophilia, expressed as log*P* values. Specially, data from alkylic side chain ester derivatives adjust to the mathematical model based on Hansch's transport theory; hence, a quantitative structure−activity relationships (QSAR) correlation with a high degree of reliance is provided. Moreover, all active compounds fit the Lipinski's rule of five. Also, the presence of additional hydroxyl groups and their derivatives in the basic skeleton does not affect the mode of action but greatly influences the activity, as they modify the transport through membranes and aqueous phases. Finally, a second hydroxyl group enhances differences of activity between alkylic side chain derivatives by increasing differences in van der Waals interactions.

KEYWORDS: Guaianolide; sesquiterpene lactones; acyl derivatives; isozaluzanin C; 5α-hydroxyisozaluzanin C; wheat etiolated coleoptile; QSAR; Hansch's transport model; Lipinski's rule of five; log*P*; lipophilia; aqueous solubility; membranes

INTRODUCTION

Lipophilia is a key factor in the absorption of any bioactive compound as it determines its bioavailability in the cell. Also, lipophilia is usually expressed through the logarithm of the octanol−water partition coefficient (log*P*) (*1*, *2*) and is present in many quantitative structure−activity relationships (QSAR) equations because an optimum equilibrium between water, the usual carrier, solubility, and lipophilia (measuring of the ability to cross cell membranes) must exist. Hence, log*P* is an important parameter in the design of pesticides because it is related with the transport of the xenobiotics in plants (*3*−*5*). The transport of substances dissolved in water in higher plants takes place essentially through xylem or phloem. A prerequisite for efficient transport of xenobiotics on xylem is an adequate water solubility (*6*), correlated with log*P* < 4. On the other hand, for an efficient basipetal transport of xenobiotics in the phloem, the water solubility of the active substance must be higher (log*P* values close to zero). Consequently, highly lipophilic substances (log*P* > 4) will stand virtually no chance of systemic transportation. Conversely, active substances with a very low lipophillicity (log*P* < 0) will not be able to cross membranes and will require other routes for entry and/or special additives in their formulation (*7*).

The importance of lipophilia is well-known and widely used in pharmacology and pharmacognosy studies and has been recently highlighted in the publication of the so-called "Lipinski's rule of five" (*8*). Using computational methods to study those factors affecting the activity, the rule predicts poor absorption and permeation for those compounds having more than five H-bond donors, ten H-bond acceptors, a molecular mass greater than 500 Da, and a calculated log*P* greater than five. Those compounds outside of the limits of this rule are supposed to be poorly active or completely inactive.

To study the influence of the lipophilic/hydrophilic characteristics on the phytotoxic activity of sesquiterpene lactones, we have used a guaianolide model according to the following requirements: simplicity in the chemical transformations and high yield and fast reactions in order to obtain high amounts of compound in a reasonable time. Guaianolides, and the closely structurally related pseudoguaianolides, constitute two highly bioactive groups of sesquiterpenes with a broad range of biological activities. They have been known for a long time to show important cytotoxicity, which has been related to their capability to undergo alkylating Michael addition reactions with biological nucleophiles, which directs the cells into apoptosis (*9*). Also, guaianolides and pseudoguaianolides are frequently cited as allelopathic agents, especially in plants of the family Compositae (*10*), and the large number of phytotoxic antecedents for this group makes them a good starting point to study

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their application as leads for developing new herbicides. However, despite the increasing number of cited references about the phytotoxic activity of natural products and structure−activity relationships (SAR) studies, no QSAR studies have been achieved so far. Moreover, only recently have some QSAR studies on the cytotoxic activity of helenanolide type (pseudoguaianolides) sesquiterpene lactones been reported (*11*, *12*). To date, no QSAR studies on phytotoxic or allelopathic activity of sesquiterpene lactones or other allelopathic agents have been reported. Only QSAR studies on the sorghum allelochemical quinone sorgoleone, reporting that it is an effective competitor of plastoquinone for the QB-binding site of PSII, have been previously published (*13*). QSAR studies are common in other fields of research, such as pharmacology or herbicides development (*14*); yet, this approach has not been achieved with allelopathic natural products. This study applies QSAR to such compounds.

MATERIALS AND METHODS

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded using $CDCl_3$ as the solvent in a Varian INOVA spectrometer at 399.952 and 100.577 MHz, respectively. The resonances of residual chloroform for ^{1}H and ^{13}C were set to δ_H 7.25 ppm and δ_C 77.00 ppm, respectively, and used as internal references. Mass spectra were obtained in a VG 1250 or a VG AUTOSPEC instruments at 70 eV, and IR spectra were recorded on a Mattson 5020 spectrometer. Column chromatography (CC) was performed on silica gel (35−75 mesh), and thin-layer chromatography analysis was carried out using aluminum-packed precoated silica gel plates. High-performance liquid chromatography (HPLC) analyses were carried out using a LiChrosorb silica 60 column and a differential RI detector in a Hitachi L-6020 HPLC instrument. All solvents were spectroscopic grade or distilled from glass prior to use.

Starting Materials. Dehydrocostuslactone was used as starting material for the synthesis of all derivatives presented in this study. It was obtained from crude Costus Resin Oil (*Saussurea lappa* commercial root extract) (Calchauvet, Seillans, France) by previous CC separation and then purified by crystallization from hexane/ethyl acetate mixtures. Isozaluzanin C [3α-hydroxyguaia-4(15),10(14),11(13)-trien-12,6-olide, **1**] and 5α-hydroxyisozaluzanin C [3α,5α-dihydroxyguaia-4(15),10(14),-11(13)-trien-12,6-olide, **18**] were prepared as previously reported (*15*). All ester derivatives were prepared according to the experimental procedures listed below.

General Acetylation Method. One hundred milligrams of each SL was dissolved in dry pyridine (10 mL), and an excess of acetic anhydride was added. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was diluted in ethyl acetate and washed with 0.1 N aqueous HCl (*3*) to remove pyridine. The organic phase was dried over anhydrous sodium sulfate, and the solvent was evaporated under vacuum, yielding the pure acetylated derivatives in high yield (>99%).

General Acylation Method. One hundred milligrams of each sesquiterpene lactone was dissolved in dry pyridine and kept in a Dewar flask at 0 °C. While the solution was stirring, 1.5 equiv of the corresponding acyl chloride was added dropwise. After 10 min, the reaction mixture was kept at room temperature overnight and worked-up as above, yielding the acylated derivatives (>98%).

Preparation of Tigloyl and Angeloyl Esters. 2,4,6-Trichlorobenzoyl chloride (0.5 mmol) and triethylamine (0.5 mmol) were added to a stirred solution of tiglic (*trans*-2-methyl crotonic) or angelic (*cis*-2-methyl crotonic) acid (0.5 mmol) in dry toluene under argon atmosphere. The resulting mixture was stirred for 2 h at room temperature and then treated with 0.05 mmol of the lactone (**1** or **18**). After it was stirred for 4 days at room temperature, the reaction mixture was diluted in ethyl acetate and extracted with water (*3*). The combined organic phases were dried over anhydrous sodium sulfate, the solvent was evaporated under vacuum, and the crude of the reaction was purified by CC (hexane:ethyl acetate 4:1), yielding **16** (86%), **17** (83%), **33** (87%), and **34** (81%).

Table 1. Values of Lipophilia and Bioactivity of Compounds Tested

Isozaluzanin C Derivatives

R	compd	IALog*P*	CLog*P*	EC_{50} (μM)
H	SM[a]	2.08	1.20	
OH	1	1.11	error	803
OAc	2	1.83	3.45	150
OPr	3	2.38	4.56	83.8
OBu	4	2.92	5.67	75.2
O*i*Bu	5	2.79	error	28.3
OVal	6	3.43	6.78	67.9
O*N*val	7	3.40	error	48.9
OHex	8	4.98	7.89	50.8
OHep	9	5.56	8.90	70.7
OOct	10	6.12	9.01	89.1
OLau	11	7.96	3.45	263
OPal	12	9.03	7.85	465
OBz	13	4.09	error	59.6
OCin	14	4.74	error	NA
O-HCin	15	5.52	1.2	264
OTig	16	4.40	error	84.9
OAng	17	4.40	error	59.3

5α-Hydroxyisozaluzanin C Derivatives

R	compd	IALog*P*	CLog*P*	EC_{50} (μM)
H	SM[a]	1.38	error	
OH	18	1.04	3.45	1850
OAc	19	1.48	4.56	163
OPr	20	1.94	5.67	123
OBu	21	1.79	error	140
O*i*Bu	22	2.38	6.78	218
OVal	23	2.31	error	85.5
O*N*val	24	2.87	7.89	181
OHex	25	1.38	error	111
OHep	26	5.56	9.01	90.3
OOct	27	7.57	3.45	138
OLau	28	8.82	7.89	NA
OPal	29	9.03	error	NA
OBz	30	2.97	error	192
OCin	31	4.79	error	30.9
O-HCin	32	1.88	error	152
OTig	33	1.88	error	134
OAng	34	5.56	9.01	65.2

[a] Starting material (SM); IALOGP, algorithm for log*P* calculation based on neural networks (cf. *18*); CLOGP, algorithm for log*P* calculation developed by Leo and Hansch (cf. *19*); NA, nonactive.

Calculation of EC_{50} and log*P*. The calculation of EC_{50} values (**Table 1**) was made by fitting the data to a dose−response sigmoidal curve with variable slope using PRISMA 4.0 package (*16*), similar to those used in herbicide studies (*17*). Log*P* was calculated using the software IALOGP (*18*) (**Table 1**) based on artificial neural networks and CLOGP (*19*) (**Table 1**).

Molecular Modeling. Structures were minimized by using the MMX force field incorporated in the Chem3D package program. To obtain theoretical molecular properties, further semiempiric minimization with MOPAC (Chem3D) was carried out. For semiempiric calculations, the PM3 method and parameters PRECISE and GEO-OK were used. Theoretical $\Delta H_f°$ values; highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), and LUMO + 1 energy values; and molecular orbital (MO) shapes were produced with MOPAC and allowed the discussion of bioassay data.

Bioassays. Coleoptiles were obtained from 3 day old wheat seedlings sown on 15 cm diameter Petri dishes fitted with Whatman no. 1 filter paper and grown at 24 °C in the dark. The etiolated seedlings were removed from the dishes and selected for size uniformity under a green safelight. The selected etiolated seedlings were placed in a Van der Wij guillotine, and the apical 2 mm was cut off and discarded. The next 4 mm portions were selected for bioassay and kept in an aqueous nutritive buffer for 1 h before being used to synchronize the growth.

Products were purified (+99%) by HPLC previous to the bioassay and tested at 1000 μM to 10 μM in a buffered nutritive aqueous solution (citric acid−sodium hydrogenphosphate buffer, pH 5.6; 2% sucrose). Mother solutions of pure compounds were prepared in dimethyl sulfoxide (DMSO) and diluted to the proper concentration with the buffer to a 0.5% v/v DMSO final maximum concentration. The following dilutions were prepared maintaining the same buffer and

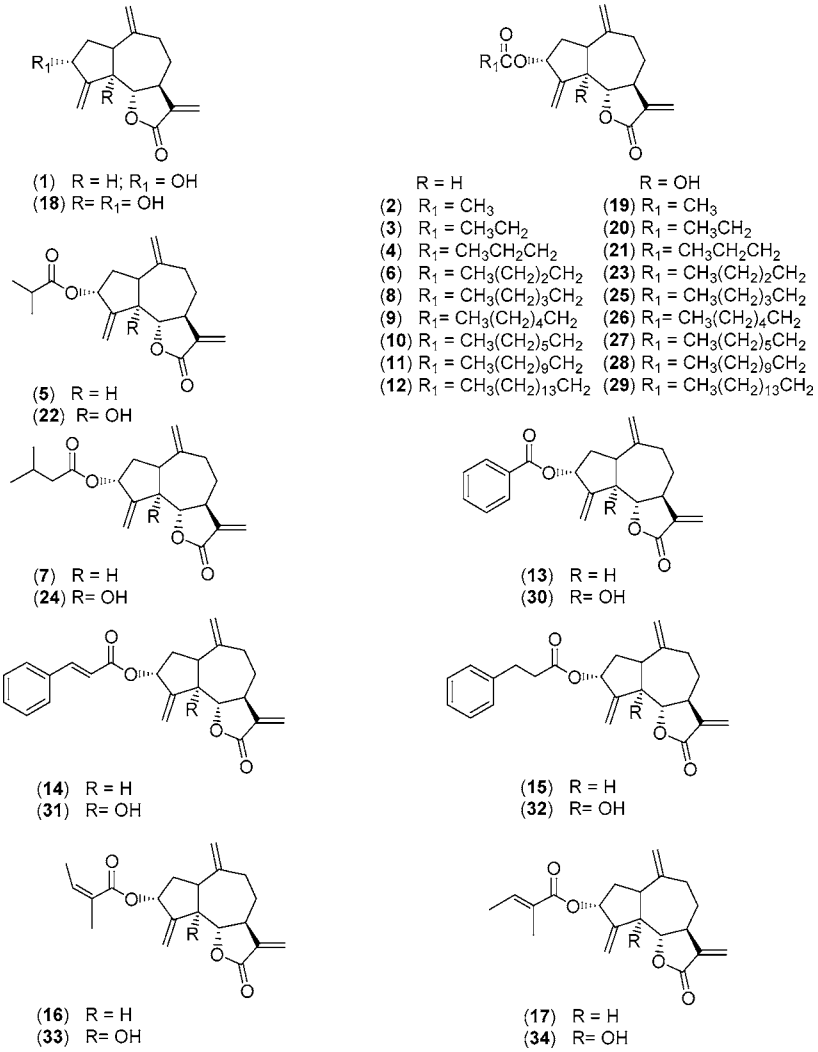

Figure 1. Structures of guaianolides tested.

DMSO concentrations. Bioassays were performed in 10 mL test tubes as follows: five coleoptiles were placed per tube containing 2 mL of test solution each; three replicates were prepared for each test solution, and the experiments were run in duplicate. Test tubes were placed in a roller tube apparatus and rotated at 6 rpm for 24 h at 22 °C in the dark. Increments of coleoptile elongation were measured by digitalization of their photographic images, and data were statistically analyzed.

RESULTS

Characterization of Compounds. The structures of all compounds were confirmed by spectroscopic methods (IR, HRMS, ^{1}H and ^{13}C NMR; see Supporting Information).

Bioassays. In general, the different ester derivatives (**Figure 1**) presented lower EC_{50} values than the free hydroxyl compounds (**Table 1**). Higher activities were obtained for alkylic side chains, while aromatic and unsaturated esters were less active but still higher than the parent compounds **1** and **18**. Long side chain lauroyl (**28**) and palmitoyl (**29**) esters were inactive (**Figure 2**).

Isozaluzanin C Derivatives (Compounds 1−17). Differences in the activity among compounds depended on the nature of the side chain. The EC_{50} values clearly showed that aliphatic derivatives **2**−**10** (**Figure 1**) have higher activities than the starting free hydroxyl product isozaluzanin C (**1**), the long side chain lauroyl (**11**) and palmitoyl (**12**) ester derivatives being the less active compounds. Aromatic and unsaturated side chain derivatives (**13**−**16**) showed moderate values, but in almost all cases, they were more active than the reference guaianolide **1** (**Figure 2**). Angeloyl derivative **17** was the most active compound in this subgroup while cinnamoyl derivative **14** was inactive.

5α-Hydroxyisozaluzanin C Derivatives (Compounds 18−34). As for the previous series, alkylic side chain derivatives presented higher activities than the reference lactone 5α-hydroxyisozaluzanin C (**18**) (**Table 1**). With the exception of the inactive lauroyl (**28**) and palmitoyl (**29**) esters, and the moderately active cinnamoyl derivatives, **31** and **32**, the rest showed high values of activity, up to 90% inhibition at 500 μM (**Figure 2**).

Log *P*. Values of log*P* were calculated using the algorithms IALOGP and CLOGP. IALOGP was chosen for this study as the commonly used algorithm CLOGP failed several times to give coherent results. Data generated by both algorithms are presented in **Table 1**.

DISCUSSION

Isozaluzanin C Esters. A positive correlation between the EC_{50} of compounds **2**−**17** and their respective log*P* values could be found in the form of a second-order polynomic curve. However, this correlation was not significant ($R^2 = 0.6347$, eq 1):

$$\log(1/EC_{50}) = -0.0322(\log P)^2 + 0.2383\log P - 2.2549;\ n = 16,\ R^2 = 0.6347 \quad (1)$$

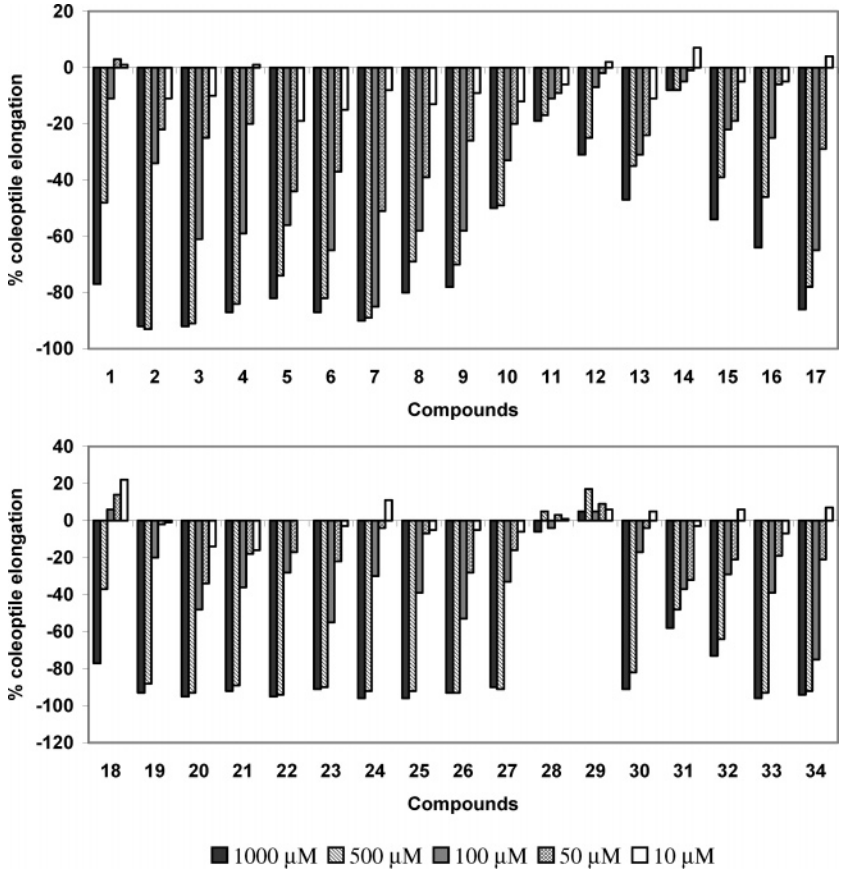

Figure 2. Effects of compounds **1**–**34** on wheat coleoptiles elongation.

Regardless of the low coefficient obtained, the correlation found was good enough to continue the study. The structural diversity of compounds tested could be the reason for such a low significance, as factors other than lipophilia might be contributing to the changes in the activity. Consequently, compounds were split into two different subgroups, alkylic (set A, compounds **2**−**12**) and unsaturated (set B, compounds **13**−**17**) olefins, following a common methodology used in medicinal chemistry (*20*). In this case, the result of the correlation between EC_{50} and log*P* with the alkylic side chains (compounds **2**−**12**, set A) gave a very good regression coefficient ($R^2 = 0.8236$, eq 2A) and even better when the compound **5** having a branched side chain was excluded from the regression ($R^2 = 0.9479$, eq 2B) (**Figure 3**), thus demonstrating the value of this approach.

$$\log(1/EC_{50}) = -0.0454(\log P)^2 + 0.3883\log P - 2.553; \quad n = 11, R^2 = 0.8236 \quad (2A)$$

$$\log(1/EC_{50}) = -0.0473(\log P)^2 + 0.4222\log P - 2.6998; \quad n = 10, R^2 = 0.9479 \quad (2B)$$

Hansch's nonlinear model (*21*) proposes that the changes in the activity of any series of structurally related compounds can be expressed as a mathematical function of transport phenomena and the intrinsic activity of the compound (eq 3). The intrinsic activity of the compound depicts its ability to bind the target site and the factors that affect the reaction between the target and the drug, expressed as a summation of electronic (E) and steric (S) parameters. Bioavailability is described in terms of lipophilia and measured through log*P*. In the classical nonlinear

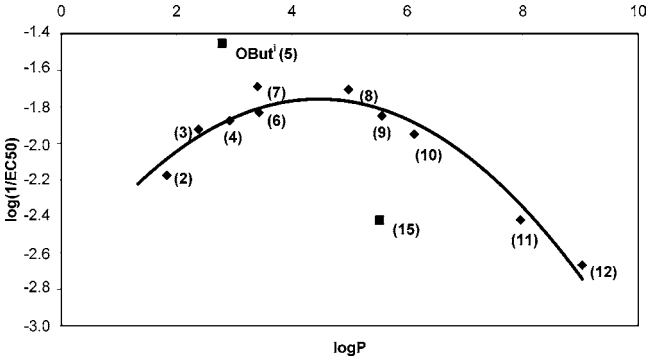

Figure 3. QSAR correlation of log(1/EC_{50}) vs IALog*P* obtained for alkylic esters **2**–**12** (set A, eq 2b; compound **5** is excluded—see text): log(1/EC_{50}) = –0.0473(log*P*)^2 + 0.4222log*P* – 2.6998; $R^2 = 0.9479$. Note how the dihydrocinnamoyloxy derivative **15** does not fit the correlation, as factors other than lipophilia influence the activity (see text). Consequently, compound **15** is not included in the regression.

model, the transport phenomena is usually expressed as a quadratic dependence of the transportation parameter log*P*.

$$\text{activity} = F(\text{transport} + \text{receptor linking}) = k_1(\log P)^2 + k_2(\log P) + k_3 \cdot \text{E} + k_4 \cdot \text{S} \quad (3)$$

Equations 2A,B suggested that changes in the activity of aliphatic derivatives (set A, **2**−**12**) fit a log*P*-dependent quadratic mathematical model. Consequently, those terms referring to electronic and steric factors must remain constant, or their changes were not as important as transport phenomena. This implied that the acylation of the C-3 hydroxyl group or the size of the side chain ester did not affect the intrinsic activity

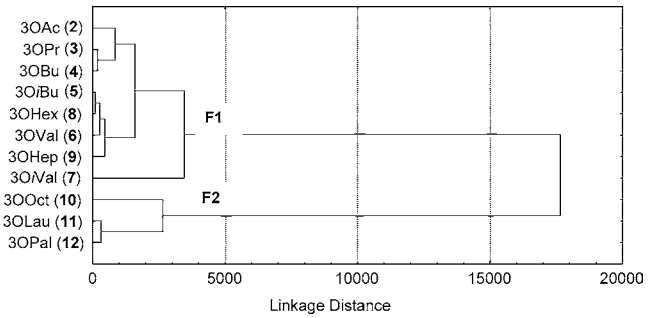

Figure 4. Cluster analysis of alkylic compounds **2**–**12**. F1, very active compounds; F2, nonactive compounds. The active compounds are placed in a decreasing order of activity around the most active esters 3O*i*Bu (**4**) and 3OHex (**8**).

of the different acyl derivatives, unless the compound surpassed the limits of log*P* and/or MW fixed by Lipinski's rule of five (*8*). Probably, this hydroxyl group is not directly involved in the link to the receptor at the site of action and its modification does not affect the intrinsic reactivity of the molecule. As a consequence of this parabolic mathematical model, those molecules with a low log*P* coefficient will partition slowly from water into a lipid membrane. If the receptor is within or beyond that membrane, they will have a low probability of reaching it in the time interval under study. Conversely, highly lipophilic molecules will be retained from the first membrane lipidic bilayers and their crossing toward the site of action will be slowed. Hence, optimal transport conditions were clearly achieved by molecules of intermediate partition coefficient, with the transfer step not being too low. This is particularly true in simple systems as coleoptiles, where the number and type of membranes to be crossed is small and different from plantules and where no water transport through any xylem and/or phloem exists. In the aliphatic derivatives series, this optimum mathematically corresponded to the apex of the parabolic function and was achieved close to log*P* = 4.4 (log*P* = 4.28 for eq 2A and logP = 4.46 for eq 2B). This point is close to the value of compound 3α-hexanoylisozaluzanin C (**8**) (log*P* = 4.98), which had the lowest EC_{50} (50.8 μM) of the entire linear side chains group. These results were in good accordance with previous studies where the hexanoyl ester has been reported as the most active derivative of a homologous series of photosynthetic inhibitors (*22*).

Branched derivatives isobutyroyl (**5**) and isovaleroyl (**7**) showed the lowest EC_{50} values, being the most active compounds of the whole series. However, compound **5** was the point that correlated worse from the whole set of alkylic compounds (**Figure 3**). Note also that compound **15** represents an intermediate situation between alkylic and unsaturated derivatives; this particular case is not included in the correlation and is discussed below. Cluster analysis confirmed these results (**Figure 4**), as active compounds in F1 subclass were grouped around the most active compounds **5** and **8** decreasing by number of carbon atoms, while 3α-isovaleroylisozaluzanin C (**7**) was grouped apart within this subclass. Nonactive compounds were grouped in a different class, F2. The differentiated activity of some branched type derivatives has been previously reported (*23*, *24*), and it is probably related to the steric factor S, thus distorting the goodness of the equation. However, further studies with a larger number of derivatives are necessary to explain these results.

Regarding the aromatic and olefinic derivatives (set B, compounds **13**−**17**), the activities shown by these compounds were generally lower than those obtained for the aliphatic compounds. However, it was not possible to obtain any good correlation between the log*P* and the activity, neither with the size of the side chain nor with the molecular volume values (data not shown). Consequently, if lipophilia is not the main factor governing the changes in the activity in this set of compounds, neither steric factors such as the size or the volume of the side chain, it must be electronic factors that were responsible for the lower activities obtained. On the other hand, the main difference between this group and the alkylic side chain group was the presence of a second π-system. Otherwise, the activity of sesquiterpene lactones has been usually related with their capability to undergo alkylating Michael addition reactions with biological nucleophiles (*25*, *26*), which directs the cells into apoptosis (*9*). The MO theory says that the reaction between two compounds takes place through the interaction of the HOMO and the LUMO. If sesquiterpene lactones are thought to behave as Michael acceptors, the MO involved in such a reaction is the LUMO of the sesquiterpene lactone and the HOMO of the bionucleophile, which provides the electrons. Isozaluzanin C (**1**) and its alkylic derivatives (set A, **2**−**12**) presented their LUMO located in the α,β-unsaturated lactone ring (**Figure 5**), and the same was also true for the dihydroxylated 5α-hydroxyisozaluzanin C (**18**) and its alkylic derivatives (**19**−**29**). This was not the case of the aromatic and unsaturated esters (compounds **13**, **14**, **16**, and **17**) where the LUMO was located in the side chain (**Figure 5**).

The benzoyl and cynnamoyl esters **13** and **14** had their LUMO located in the aromatic side chain. Also, a lower energy than those of the alkylic series was obtained in the theoretical calculations (**Table 2**). The following unoccupied MO (LUMO + 1) was located in the lactone ring and presented similar energy to the LUMOs of the alkylic esters (**Table 2**). Consequently, any Michael reaction involving a nucleophile should fill first this lower energy MO. However, in the case of the benzoic ester **13**, this reaction is not possible as it involves an addition of the bionucleophile to an aromatic ring without any electrophilic chararacteristic. Then, the MO involved should be the LUMO + 1. Moreover, the nucleophilic addition to the carbonyl group of the benzoic ester competes with the Michael addition to the lactone ring and shifts the reactivity of the molecule to a different zone (the cyclopentane ring) and, thus, the interaction with the bionucleophile. Hence, a lower reactivity should be expected, and this theory correlates well with the lower bioactivity observed in the benzoic ester **13**.

In the case of the cinnamoyl ester **14**, the situation was repeated, but the consequences were different; again, the LUMO was located in the side chain and the energy was even lower than in the previous case, thus indicating a higher reactivity. In this case, a Michael addition to the α,β-unsaturated carbonyl system is possible. Consequently, the reactive zone of the molecule has changed from the lactone ring to the side chain. If the molecule presents a fixed orientation in the active site, it is possible that the bionucleophile reactive group is placed in such a way favoring the reaction with the double bond in the lactone ring. Compound **14** was totally inactive and the lack of bioactivity could be explained through a lack of reactivity caused by the change in the orientation of the reactive zones in the molecule. Furthermore, if the addition reaction could take place through the energetically favored cinnamoyl ester, the bulky phenyl group avoids an easy access, thus disfavoring the adduct formation. This was further confirmed by the activity obtained with the dihydroderivative of the cinnamic acid (**15**), which was

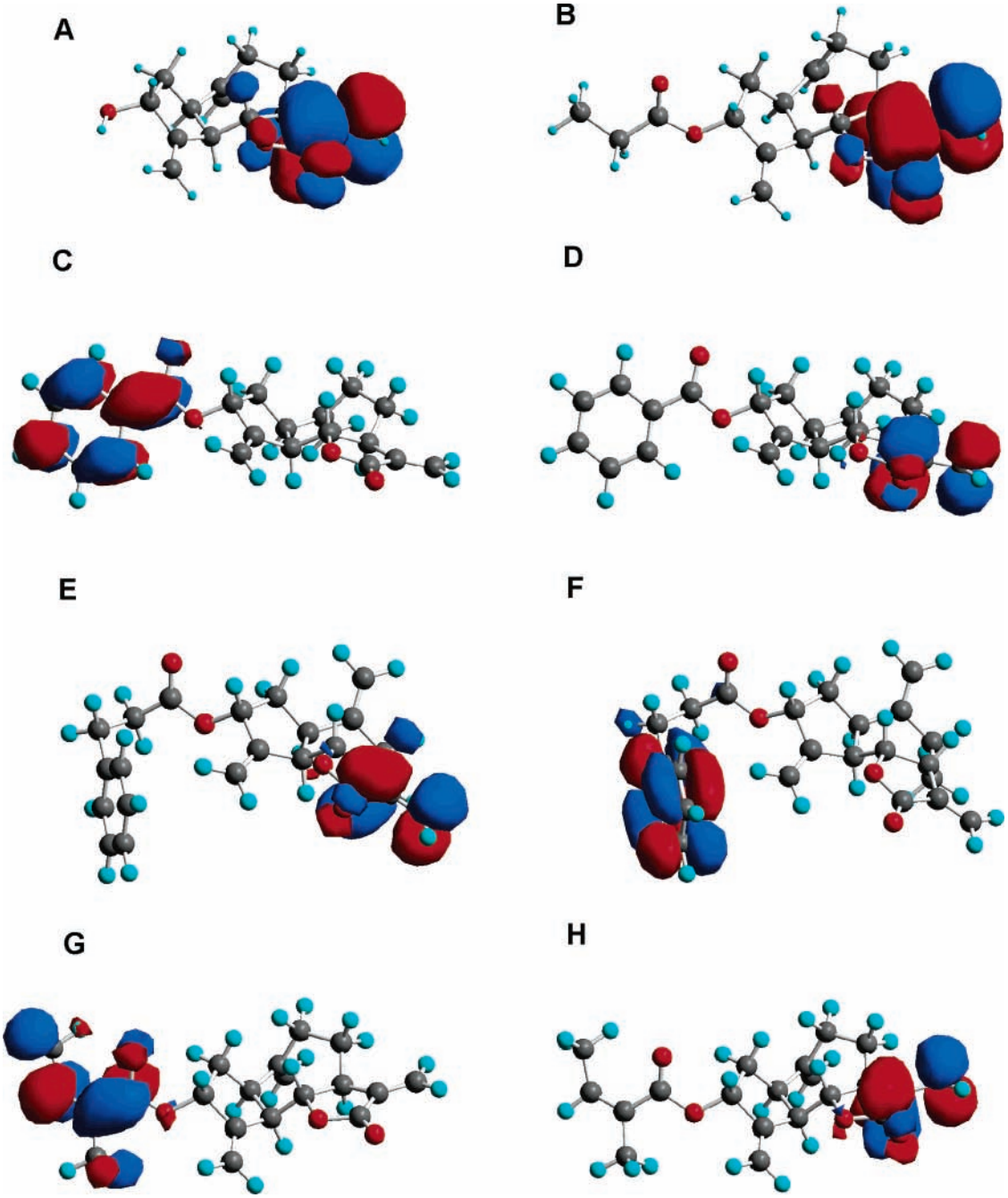

Figure 5. MOs involved in the Michael addition of a bionucleophile to the SL tested. Isozaluzanin C (**1**): (**A**) LUMO (E = –0.224994 eV); propionyloxyisozaluzanin C (**3**): (**B**) LUMO (E = –0.22079 eV); benzoyloxyisozaluzanin (**13**): (**C**) LUMO (E = –0.696346 eV) and (**D**) LUMO + 1 (E = –0.196523 eV); dihydrocinnamoyloxyisozaluzanin C (**15**): (**E**) LUMO (E = –0.968833 eV) and (**F**) LUMO + 1 (E = –0.233009 eV); tigloyloxyisozaluzanin C (**16**): (**G**) LUMO (E = –0.210318 eV) and (**H**) LUMO + 1 (E = –0.198057 eV).

again active. In this case, the LUMO was located in the lactone ring and its energy was similar to those of the alkylic side chains (ca. −9.908813 eV) (**Table 2**). Consequently, the reactivity was similar to that in the alkylic series, but its log$P$ value did not fit the QSAR equation obtained for them, probably due to steric factors caused by the aromatic ring.

Finally, tigloyl (**16**) and angeloyl (**17**) esters constituted, again, a different case. Both compounds presented two α,β-unsaturated esters, one in the lactone ring and the other in the side chain. Their chemical behavior was quite similar, as exemplified by the similar energies obtained for the LUMO and the LUMO + 1 MOs (**Table 2**). Consequently, their reactivity with the bionucleophile should be quite the same, and so the bioactivity observed. Data obtained correlated well with this hypothesis (**Table 1**).

5α-Hydroxyisozaluzanin C Derivatives. On the basis of the results obtained for the monohydroxylated derivatives series, a similar analysis splitting data into the same subgroups (linear and unsaturated) was done. Analogue compounds behaved approximately the same and, again, lauroyl (**31**) and palmitoyl (**32**) esters did not fit the Lipinski's rule of five and, consequently, they were inactive. The correlation between log$P$ and EC_{50} with the rest of the linear derivatives is shown in **Figure 6**. The most significant difference with the previous results (**Figure 3**) was a zigzag like disposition of data and a nonsignificative correlation (R^2 = 0.5871). A careful examina-

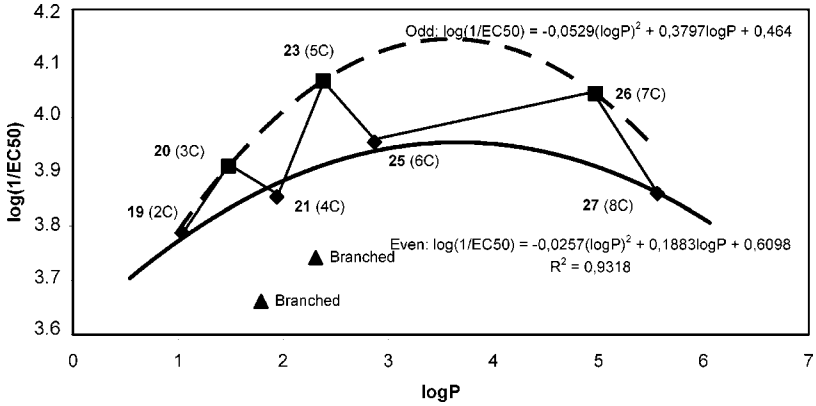

Figure 6. Log*P* vs log(1/EC_{50}) of compounds **19**–**27**. Note how those compounds having an alkylic side chain with an even (◆) or an odd (■) number of carbons adopt a zigzag like disposition. Branched derivatives (▲) do not fit any correlation.

Table 2. Values of Energy of the MOs HOMO, LUMO, and LUMO + 1, as Calculated by MOPAC and Using the Algorithm PM3[a]

compd	R	HOMO (eV)	LUMO (eV)	LUMO + 1 (eV)
isozaluzaninC (**1**)	OH	–10.119687	–0.224994	0.640875
2	Ac	–10.127291	–0.222138	0.542805
3	Pr	–10.127514	–0.22079	0.541565
4	But	–10.120613	–0.222994	0.544275
5	*i*But	–10.12835	–0.22289	0.525068
6	Val	–10.121449	–0.223647	0.54496
7	iVal	–10.130105	–0.223264	0.477537
8	Hex	–10.129968	–0.22273	0.528653
9	Hep	–10.142059	–0.224308	0.512638
10	Oct	–10.127365	–0.223558	0.54037
11	Lau	–10.042477	–0.467164	0.484769
12	Pal	–10.146141	–0.305457	0.561069
13	Bz	–10.128305	–0.696346	–0.196523
14	Cin	–9.607365	–0.968833	–0.233009
15	HCin	–9.908813	–0.23573	–0.058118
16	Tig	–10.107169	–0.210318	–0.198057
17	Ang	–9.978424	–0.208833	–0.183761
5α-hydroxy-isozaluzanin C (**18**)	OH	–10.133996	–0.236815	0.558543
19	Ac	–10.139576	–0.234296	0.44798
20	Pr	–10.141792	–0.237533	0.430697
21	But	–10.140302	–0.237529	0.449424
22	*i*But	–10.143028	–0.237628	0.430011
23	Val	–10.143177	–0.236805	0.424823
24	iVal	–10.14109	–0.237319	0.404731
25	Hex	–10.080745	–0.241896	0.470843
26	Hep	–10.145822	–0.236972	0.445843
27	Oct	–10.178755	–0.251855	0.338566
28	Lau	–10.146026	–0.242956	0.380455
29	Pal	–10.059412	–0.247194	0.474537
30	Bz	–10.146121	–0.443008	–0.240987
31	Cin	–9.606749	–0.9675	–0.255434
32	HCin	–9.937339	–0.252506	–0.081891
33	Tig	–10.137151	–0.238409	0.023132
34	Ang	–9.92593	–0.208164	–0.202724

[a] OAng derivatives MOs energies were calculated with the algorithm AM1 instead of PM3, as it did not render a planar geometry for the angeloyl side chain.

tion showed that the esters with an even number of carbon atoms were on top and odd number side chains were at the bottom of the zigzag shape. However, when separate analyses for odd and even carbon series (equations 4 and 5) were performed, a significant correlation was found for the even carbon series. Of course, in the case of derivatives with an odd number or carbons in the side chain, a quadratic correlation with three points mathematically has a $R^2 = 1$. However, the good correlation obtained for even derivatives permits considering the goodness of this approach.

Even derivatives (compounds **19**, **21**, **25**, and **27**):

$$\log(1/EC_{50}) = -0.0257(\log P)^2 + 0.1883\log P + 0.6098;\ n = 4,\ R^2 = 0.9318 \quad (4)$$

Odd derivatives (**20**, **23**, and **26**):

$$\log(1/EC_{50}) = -0.0529(\log P)^2 + 0.3797\log P + 0.464;\ n = 3,\ R^2 = 1.000 \quad (5)$$

The zigzag effect is observed also in some physical properties of carboxylic acid and derivatives, such as melting and boiling points (*27*) and is related with the van der Waals forces. Even members of a homologous series could be packed better than odd members or vice versa. Consequently, in one of the two series, higher van der Waals forces will be exerted, thus yielding higher molecular interactions expressed as higher melting or boiling points. In our case, two different hypotheses are given to explain this zigzag shape of data: one related with transport through the membranes and the other based on the interaction between the lactone and the active site.

Transport Hypothesis. The macroscopic effects observed are related mainly with transport phenomena. Cell membranes are formed by a lipidic bilayer, essentially impermeable to most ionic and polar substances, and specific transport proteins are inserted across them and responsible for most of their transport properties (*28*). However, low polarity allelochemicals might not be recognized by these specific proteins nor need them to cross the membranes. Their low molecular masses and polarity might allow them to be dissolved into the lipidic bilayer, thus allowing their penetration through it. Fatty acids with an even number of carbon atoms (usually 16−20) are constituents of eukaryotic cell membranes, so it is possible that low polarity molecules with linear side chains of different lengths interact with them during the membrane crossing step or even with hydrophobic regions of membrane protein if active transport takes place. If compounds having an even number of carbon atoms in the side chain are better packed with these hydrophobic areas than odd ones, the membrane crossing will slow, thus diminishing the bioavailability of the compound.

Isozaluzanin C is an almost planar molecule, with a boatlike conformation and the lactone ring lying almost in the same plane. Both faces are mainly the same, with the exomethylene groups in a beta (above the plane) disposition. In this case (compounds **2**−**17**), differences in the packing are not big enough to exert any observable zigzag effect in the bioactivity (**Figure 3**). However, the asymmetry induced in the α face by the hydroxyl group in the 5α-hydroxyisozaluzanin C series

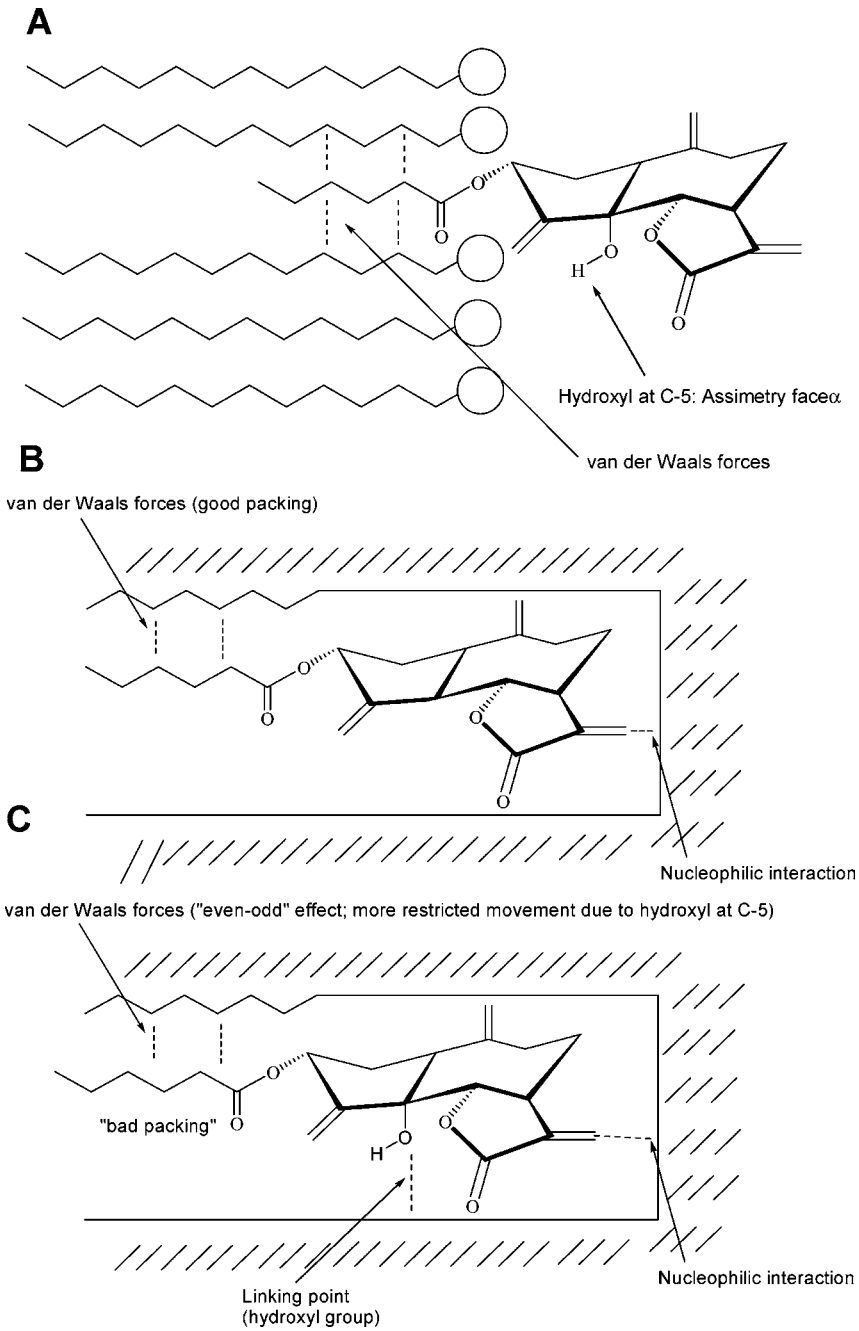

Figure 7. (**A**) Interaction with the cell membranes: The side chain of the SL establishes van der Waals forces with the hydrophobic tails of the fatty acids; the introduction of a hydroxyl group in the lower face of the SL induces the even–odd effect. (**B**,**C**) Interaction with the active site: The side chain of the SL interacts through van der Waals forces with a hydrophobic region of the active site (**B**); the introduction of another hydroxyl group (**C**) lowers the degree of freedom and enhances differences in the packing of the side chain and the active site giving rise to the even–odd effect.

(compounds **18**−**34**) can explain the marked "even−odd" effect observed with the EC_{50}: one of the two series will pack better than the other due to restrictions caused by the hydroxyl group (**Figure 7A**). These differences should be present also in the isozaluzanin C series (**2**−**17**), but the presence of the hydroxyl group at C-5 in the second series (**18**−**34**) will enhance this effect.

Site of Action Hypothesis. The effects observed could be a superposition of transport effects (parabolic relationship) and intrinsic activity of the compound (zigzag shape). Although the site of action is yet unknown, the existence in its surroundings of a hydrophobic region that interacts with the hydrophobic side chain could be responsible of the overlapped even−odd effect through van der Waals interactions. Although van der Waals forces are extremely weak, when the hydrophobic region is large and a correct spatial arrangement exists, they are able to stabilize the molecule−receptor complex. When a hydroxyl group at C-5 is not present, the side chain packs better with this hydrophobic region and a high activity is observed (set A). On the other hand, the hydroxyl group at C-5 introduces a second linkage point in the molecule and the freedom of the system is lower. In this case, movements on the active site are restricted and one of the two series packs better, thus giving rise to the even−odd effect (**Figure 7B**,**C**).

From these results, we can conclude that transport phenomenon and van der Waals interactions are the most influencing factors in the activity of these derivatives since they fit Hansch's model. Transport phenomena represent the general frame and

van der Waals interactions might resemble packing between the molecule and the hydrophobic regions presented in the living system, thus giving rise to the even−odd effect.

Branched derivatives **22** and **24** were not the most active compounds of the series, opposite to compounds **5** and **7**, even though they present good EC_{50} values [218 (**22**) and 181 (**24**) μM] (**Table 1**). However, opposite to the isozaluzanin C derivatives group of compounds, they do not fit any of the two correlations. Regarding aromatic and olefinic derivatives, their behavior is similar to the monohydroxylated group of sesquiterpene lactones. The main difference arose from the couple tigloyloxy and angeloyloxy, with no important differences in the activity among them.

Isozaluzanin C vs 5α-Hydroxyisozaluzanin C Series. Compounds belonging to the isozaluzanin C series (**2**−**17**, set A) show lower EC_{50} values than those of the 5α-hydroxyisozaluzanin C set (**18**−**34**) (**Table 1**), highlighting that the hydroxyl group at C-5 plays an important role and greatly influences the activity, diminishing it when present. This is more evident when the hydroxyl group at C-3 is free, yielding the higher differences in the activity. In general, a great increase of the activity is obtained for most acyl derivatives, thus resembling the importance of an adequate lipophilia and the influence of transport.

In summary, we can conclude that an increase in the lipophilia turns on a general enhance of the activity, thus resembling the importance of transport through membranes in the bioactivity of these compounds. Also, there is an optimum for transport determined by the apex of the QSAR equation obtained that adjusts to the Hansch's nonlinear model; beyond that point, the bioactivity starts again to decrease, as a too strong interaction between the lipidic bilayer and the compound is established, retaining the compound in the membranes. The fact that the bioactivity depends only on lipophilia also means that the mode of action of all of these compounds is the same. In other words, the introduction of a side chain does not affect the interaction with the active site, excepting to enhance differences between side chains giving rise to the even−odd effect in the 5α-hydroxyisozaluzanin C esters.

Regarding aromatic and unsaturated side chains, the analysis reveals that electronic factors modify the reactivity of these set of compounds and must be taken into account along with lipophilia in the QSAR equation. However, the low number of compounds tested does not allow us to perform such a calculation and only qualitative considerations can be given to explain the results of bioactivity. Finally, it is also important to remark that active compounds fit the Lipinski's rule of five, thus demonstrating the goodness of this pharmacological approach in phytotoxicity studies to predict if a compound can or cannot be active.

ABBREVIATIONS USED

QSAR, quantitative structure−activity relationships; MO, molecular orbital; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital.

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This paper is affectionately dedicated to Professor Nikolaus H. Fischer, Mississippi State University, on his "official" retirement, with our best wishes on this new step in his personal and scientific life.

Supporting Information Available: Spectroscopic data of 34 sesquiterpene lactones tested, including IR, HRMS, and ^{1}H and ^{13}C NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Mannhold, R.; Rekker, R. F.; Dross, K.; Biljoo, G.; de Vries, G. The lipophilic behavior of organic compounds: 1. An updating of the hydrophobic fragmental constant approach. *Quant. Struct.-Act. Relat.* **1998**, *17*, 517−536.

(2) Rekker, R. F.; Mannhold, R.; Biljoo, G.; de Vries, G.; Dross, K. The lipophilic behaviour of organic compounds: 2. The development of an aliphatic hydrocarbon/water fragmental system via interconnection with octanol−water partitioning data. *Quant. Struct.-Act. Relat.* **1998**, *17*, 537−548.

(3) Sicbaldi, F.; Sacchi, G. A.; Trevisan, M.; Del Re, A. A. M. Root uptake and xylem translocation of pesticides from different chemical classes. *Pestic. Sci.* **1997**, *50*, 111−119.

(4) Inoue, J.; Chamberlain, K.; Bromilow, R. H. Physicochemical factor affecting the uptake by roots and translocation to shoots of amine bases in barley. *Pestic. Sci*. **1998**, *54*, 8−21.

(5) Satchivi, N. M.; Stoller, E. W.; Wax, L. M.; Briskin, D. P. A nonlinear dynamic simulation for xenobiotic transport and whole plant allocation following foliar application II. Model validation. *Pestic. Biochem. Physiol*. **2000**, *68*, 85−95.

(6) Jacob, F.; Newmann, S. Principles of uptake and systemic transport of fungicides within the plant. In *Modern Selective Fungicides*; Lyr, H., Ed.; Longman Scientific & Technical: Essex, United Kingdom, 1987; pp 13−30.

(7) Baker, E. A.; Hayes, A. L.; Butler, R. C. Physicochemical properties of agrochemicals: Their effects on foliar penetration. *Pestic. Sci.* **1992**, *34*, 167−182.

(8) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2001**, *46*, 3−26.

(9) Dirsch, V. N.; Stuppner, H.; Vollmar, A. M. Helenalin triggers a CD95 death receptor-independent apoptosis that is not affected by overexpression Bcl-xL or Bcl-2. *Cancer Res.* **2001**, *61*, 5817−5823.

(10) Fraga, B. M. Natural sesquiterpenoids. *Nat. Prod. Rep.* **2003**, *20*, 392−413.

(11) Schmidt, T. J. Quantitative structure-cytotoxicity relationships within a series of helenanolide type sesquiterpene lactones. (Helenanolide type sesquiterpene lactones, IV). *Pharm. Pharmacol. Lett.* **1999**, *9*, 9−13.

(12) Schmidt, T. J.; Heilmann, J. Quantitative structure-cytotoxicity relationships of sesquiterpene lactones derived from partial charge (Q)-based fractional Accessible Surface Area Descriptors (Q_frASAs). *Quant. Struct.-Act. Relat.* **2002**, *21*, 276−287.

(13) Czarnota, M. A.; Paul, R. N.; Dayan, F. E.; Nimbal, C. I.; Weston, L. A. Mode of action, localization of production, chemical nature, and activity of sorgoleone: a potent PSII inhibitor in *Sorghum* spp. Root exudates. *Weed Technol.* **2001**, *15*, 813−825.

(14) Hansch, C.; Leo, A. Exploring QSAR. *Fundamentals and Applications in Chemistry and Biology*; Heller, S. R., Ed.; American Chemical Society: Washington, DC, 1995.

(15) Macías, F. A.; Galindo, J. C. G.; Castellano, D.; Velasco, R. F. Sesquiterpene lactones with potential use as natural herbicide models. 2. Guaianolides. *J. Agric. Food Chem.* **2000**, *48*, 5288−5296.

(16) *PRISMA 4.0*; GraphPad Software, Inc.: San Diego, CA, 2003.

(17) Schabenberger, O.; Tharp, B. E.; Kells, J. J.; Penner, D. Statistical test for hormesis and effective dosages in herbicide dose−response. *Agric. J.* **1999**, *91*, 713−721.

(18) *IALOGP*; Interactive Analysis: Belford, MA, 2000.

(19) Hansch, C.; Leo, A. J. Substituent constant for correlation analysis. *Chemistry and Biology*; Wiley: New York, 1979.

(20) Kupchan, S. M.; Eakin, M. A.; Thomas, A. M. Tumor inhibitors. 69. Structure-cytotoxicity relationships among the sesquiterpene lactones. *J. Med. Chem.* **1971**, *14*, 1147−1152.
(21) Hansch, C.; Fujita, T. A method for the correlation of biological activity and chemical structure. *J. Am. Chem. Soc.* **1964**, *86*, 1616−1626.
(22) Sidoova, E.; Kralova, K.; Loos, D. Synthesis of 2-(6-acetamidobenzothiazolethio)acetic acid esters as photosynthetic inhibitors. *Molecules* **1998**, *3*, 135−140.
(23) Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. Structure−activity relationship studies of novel carbocyclic influenza neuraminidase inhibitors. *J. Med. Chem.* **1998**, *41*, 2451−2460.
(24) Rapaka, R. S.; Makriyannis, A. Structure−activity relationships of the cannabinoids. In *Research Monograph Series*; Rapaka, D. O., Makriyannis, A., Eds.; National Institute of Drug Abuse (NIDA): Rockville, MD, 1987; p 79.
(25) Galindo, J. C. G.; Hernández, A.; Macías, F. A.; Téllez, M.; Paul, R. N.; Duke, S. O. Dehydrozaluzanin C, a natural sesquiterpenolide, causes rapid plasma membrane leakage. *Phytochemistry* **1999**, *52*, 805−813.
(26) Schmidt, T. J. Helenanolide-type sesquiterpene lactones-III. Rates and stereochemistry in the reaction of helenanin and related helenanolides with sulfhydryl containing biomolecules. *Bioorg. Med. Chem.* **1997**, *5*, 645−653.
(27) *Handbook of Chemistry and Physics*, 59th ed.; CRC Press: Boca Raton, FL, 1978.
(28) Poole, R. J. Plasma membrane and tonoplast. In *Solute Transport in Cells and Tissues*. *Monographs and Surreys in the Bioscience*; Baker, D. A., Hall, J. L., Eds.; Langman Scientific and Technical: Harlow, U.K., 1988.

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