

Structure–Antifeedant Activity Relationship of Clerodane Diterpenoids. Comparative Study with Withanolides and Azadirachtin

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A structure–antifeedant activity relationship (SAR) study of clerodane diterpenoids was carried out. Attention was focused on the feeding-deterrent activities exhibited toward *Tenebrio molitor* by clerodane diterpenoids and withanolides. Azadirachtin was chosen as a reference compound. SAR studies on the clerodane compounds indicate that the stereoelectronic factors are more important than the hydrophobic aspects as determinants of antifeedant activity. A furan ring in the side chain and a carbonyl α,β -unsaturated (or *spiro*-epoxide) group appear to be indispensable for the biological response. A conformational study indicate that the optimum interatomic distance between these moieties is a range between 9.5 and 10.5 Å. In addition, a similar stereoelectronic response was found among withanolides and azadirachtin. On the basis of these results it is reasonable to imagine a closely related chemical mechanism for these compounds.

Keywords: Antifeedant activity; clerodanes; withanolides; azadirachtin; SAR; minimal structural requirements; molecular modeling

INTRODUCTION

It is well-known that plants contain insect antifeedants as well as pesticides and repellents (Hedin et al., 1994). Plants have developed highly elaborate chemical defenses against insect attacks, and these have provided a rich source of biologically active compounds that may be used as novel crop-protecting agents.

The study of allelochemical interactions between insects and plants is currently one of the most actively investigated subjects in chemical ecology, partly due to its interesting perspectives for development of new biorational pesticides of natural origin (Camps, 1988; Arnason et al., 1989). These interactions involve numerous secondary plant metabolites, which may interfere with the behavior, growth, or development of the insects.

In the present paper we report the feedant-deterrent activities exhibited toward *Tenebrio molitor* by a set of clerodane and withanolide molecules (Figures 1 and 2).

In the literature there are many reviews (Camps, 1991; La Font et al., 1989; Belles et al., 1985) reporting the “antifeedant” or “growth-inhibiting” activities of compounds structurally related to the molecules reported here. However, examination of those compilations reveals several impediments to developing a quantitative understanding of structure–activity relationships among these compounds. First, different

investigators seldom utilize the same bioassay for one species; interspecies differences in the response of the test insects can easily mask any meaningful observations of structure–activity relationships. Even where the same bioassay has been used, differences in the larval stage tested may make comparisons invalid, as neonate or early instar larvae are known to be more sensitive to antifeedant agents than later instar larvae (Champagne et al., 1989). Second, the majority of studies were designed only to detect feeding deterrence, and therefore the molecular aspects have not been discussed in detail.

In the present study we have mainly focused our attention on the feeding-deterrent activities exhibited toward *T. molitor* L. larvae by clerodane diterpenoids and withanolides. Azadirachtin was chosen as a reference compound. Thus, we have evaluated azadirachtin using the same experimental conditions.

To understand better the above experimental results, we conducted a computer-assisted conformational and electronic study on the compounds shown in Figures 1 and 2. The purpose was to obtain more precise information as to how closely these compounds resemble each other in terms of the spatial orientations of the essential components for receptor recognition.

EXPERIMENTAL PROCEDURES

Insect. A stock culture of *T. molitor* (L) (Coleoptera: Tenebrionidae) larvae was maintained on wheat bran in plastic boxes at 24 ± 1 °C with a 16:8 (L/D) photoperiod (Sosa et al., 1994). A voucher specimen is located at the Cátedra de Entomología, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina.

Test Compounds. Diterpenes **1** and **2** were obtained from aerial parts of *Baccharis crispa* Sprengel (Tonn et al., 1979;

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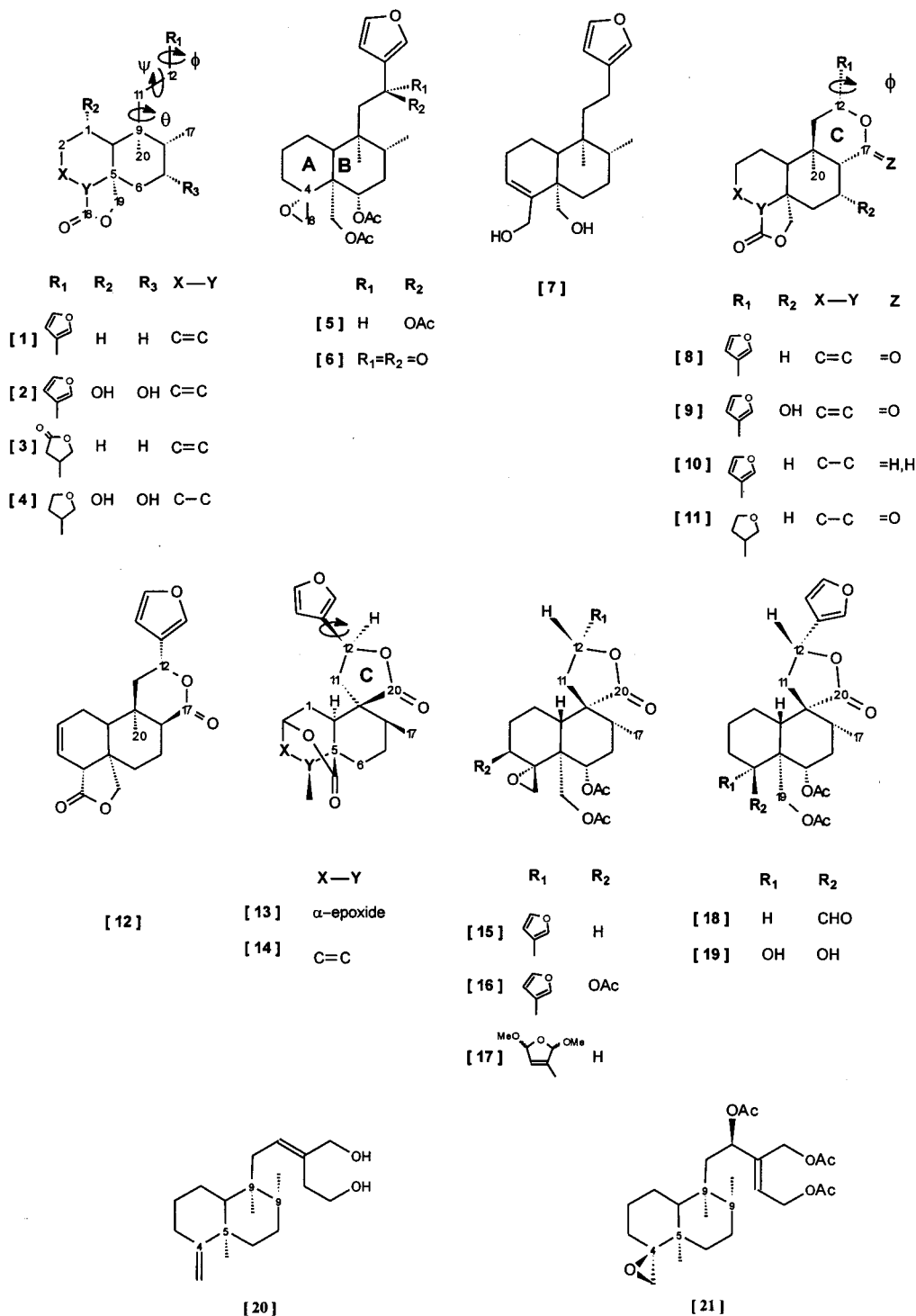


Figure 1. Key structural features of clerodane diterpenoids studied in this paper. The designations of the torsional angles in the connecting units are also included.

Tonn and Giordano, 1980). The dilactone **3** was isolated from *Baccharis triangularis* (Haumann) (Gianello and Giordano, 1989). Compound **4** was prepared from **1** by catalytic hydrogenation (Tonn et al., 1979). Compounds **5** and **15** were extracted from *Teucrium grisebachii* Hieron. (Tonn et al., 1990), and clerodane **6** was prepared from **5** using NaH/MeOH followed by Jones oxidation (Sosa et al., 1994). *neo*-Clerodane **7** was prepared from **1** by H₄LiAl/THF reduction (Gianello and Giordano, 1982). Bacchotricuneatin A **8** was isolated from aerial parts of *Baccharis rhotinodes* (Sosa et al., 1994). From *Baccharis spicata* (Lam.) Beill. was recovered 7- α -hydroxy-bacchotricuneatin A **9**; the unusual reduced derivative **10** was prepared from **8** by NaBH₄ using a mixture of 1,4-dioxane/

MeOH as solvent (Gallardo et al., 1996). Compound **11** was prepared from **8** by catalytic hydrogenation. Salviarin (**12**) was isolated from the aerial parts of *Salvia reflexa* Hornem (Nieto et al., 1996). *ent-neo*-Clerodanes **13** and **14** were obtained from *Baccharis artemisioides* H. et A. (Tonn et al., 1988, 1989).

Compound **16** was isolated in the course of the phytochemical study of *Teucrium nudicaule* H. from Chile, and the epimeric mixture of *neo*-clerodane **17** was prepared from **15** using bromine and MeOH at -25 °C. In the same study, compounds **18** and **19** were prepared from **15** by reaction with HCl(g)/Et₂O and BF₃/Et₂O complex, respectively (Gallardo et al., 1996).

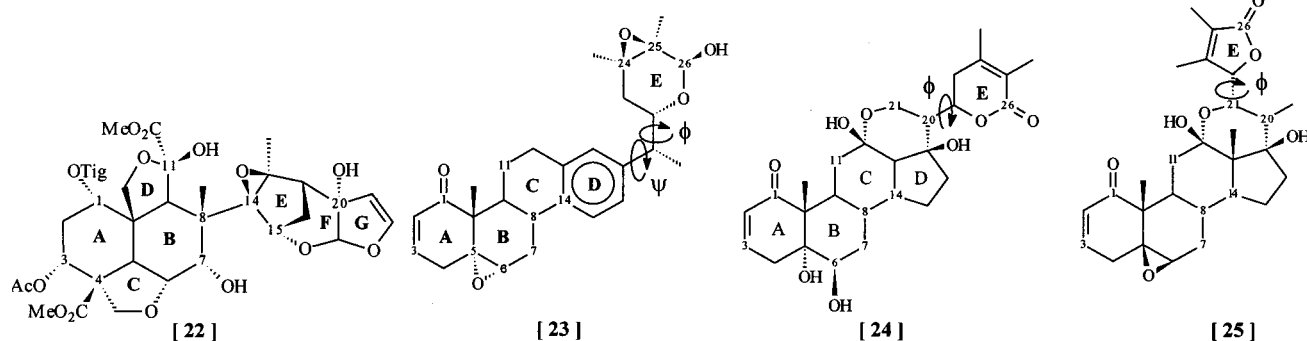


Figure 2. Structural features of azadirachtin **22** and withanolides **23–25**. Hydrophobicity constants (K_w) obtained by HPLC-RP for the respective compounds are as follows: **22**, 3.19; **23**, 5.08; **24**, 3.35; and **25**, 2.08.

(*E*)-Isolarinidiol **20** was obtained by reduction with LAH of (*E*)-isolarinidial (San Feliciano et al., 1993) from aerial parts of *Linaria saxatilis*.

Compound **21** was obtained by MCPBA epoxidation of isolarintriol triacetate (San Feliciano et al., 1985) isolated from aerial parts of *L. saxatilis* (Gordaliza et al., 1994).

Compound **22** (Butterworth and Morgan, 1968) was generously provided by Professor S. V. Ley (Imperial College, London, U.K.). Compounds **23** (Veleiro et al., 1992) and **24** (Bonetto et al., 1993) were kindly provided by Professor Oberty (Universidad Nacional de Córdoba, Argentina). Trechonolide A (**25**) was recovered from aerial parts of *Trechonaetes laciniata* (Lavie et al., 1987).

Antifeedant Bioassay. Carrot slices (2.5 cm diameter and 0.5 cm thick) were coated with 100 μ L/slice of test emulsions containing the target compounds. Emulsions were prepared at a concentration of 100 ppm in a mixture of H₂O/MeOH/Me₂CO (90:5:5) containing Triton CS-7 (0.1 vol %) as solvent (Lidert et al., 1985). The emulsions were treated by ultrasonic irradiation for 5 min. Untreated slices were coated with solvent blanks. After drying, six control slices and six treated slices were weighed and separately placed in plastic boxes with 20 third instar larvae of *T. molitor* for each test. Next, the slices were removed, reweighed, and renewed every 24 h for 10 days. Calculations of the amounts of treated or control slices eaten were made by subtracting the weight loss determined during test. The activity was expressed as a percentage of feeding inhibition (PFI) (Reed and Jacobson, 1983) according to

$$\text{PFI} = \left[\frac{\% \text{ consumed of treated slices}}{\% \text{ consumed of treated slices} + \% \text{ consumed of untreated slices}} \right] \times 100$$

This experiment was conducted in duplicate, with eight repetitions, for each of the corresponding compounds assayed. The average of the standard deviation was 4.37, and in general smaller values of PFI indicate higher antifeedant activity. Thus, taking into account the standard deviation (SD), values obtained for PFI <40 indicate antifeedant activity.

Examinations and summaries of data are based on analyses of variance (block design ANOVA) followed by means comparisons. The results are shown in Table 1.

Chromatographic Retention Measurements. The chromatographic data were obtained at room temperature with a Beckman (model 332) liquid chromatograph equipped with a variable wavelength detector. A 5- μ m C₈ (15 cm \times 4.6 mm i.d.) column was used, and the flow rate was 1 mL/min. The mobile phases consisted of different volume fractions of methanol and water. The column dead time (T_0) was estimated from the retention time of deuteromethanol measured at 220 nm with methanol as eluent. The obtained retention data (T_r) were used to derive the values of the capacity factor (K'), which was calculated in the usual manner; that is, $K' = (T_r - T_0)/T_0$. Finally, the values of $\log K'w$ were obtained by linear regression with the equation

$$\log K'\varphi = \log K'w - S\varphi$$

Table 1. PFI Obtained for the Different Compounds

| compound | PFI | compound | PFI | compound | PFI |
|----------|--------------------|-----------|--------------------|-----------|--------------------|
| 1 | 30.96 ^a | 10 | 46.00 | 19 | 45.00 |
| 2 | 31.26 ^a | 11 | 49.40 | 20 | 57.32 |
| 3 | 62.64 | 12 | 47.30 | 21 | 51.90 |
| 4 | 48.35 | 13 | 57.87 | 22 | 29.57 ^a |
| 5 | 31.45 ^a | 14 | 62.72 | 23 | 29.89 ^a |
| 6 | 57.96 | 15 | 25.03 ^a | 24 | 31.55 ^a |
| 7 | 48.70 | 16 | 30.63 ^a | 25 | 44.67 |
| 8 | 22.62 ^a | 17 | 47.30 | | |
| 9 | 26.60 ^a | 18 | 41.00 | | |

^a Compounds having PFI values <33 were considered to be active.

where φ is the volume fraction of the organic modifier in the aqueous eluent.

Computational Analysis. All of the calculations reported here were carried out using molecular mechanics and semiempirical AM1 (Dewar et al., 1985) quantum mechanics methods. Molecular mechanics (MM) calculations were performed using an empirical atom–atom potential method designed by the San Luis group (Enriz and Jáuregui, 1991; Giordano et al., 1992). For the molecular orbital semiempirical calculations the AM1 method implemented in the standard version of MOPAC 6.0 (Stewart, 1990) was used. We have previously reported the use of these methods for biological systems, with excellent results (Enriz et al., 1993, 1994a,b; Baldoni et al., 1996, 1997).

Using the "buildup" strategy, we initially considered the skeleton of the molecules stripped of all its substituents. First of all, three fragments of the full skeleton of clerodanes, ring (A + B + C) and four fragments of the skeleton of withanolides (A + B + C + D) were optimized. Subsequently, the substituents of the different compounds were each placed on the optimized skeleton, using the minimum global geometrical parameters of the substituents as obtained by the MM calculations. Finally, all of the structural parameters of the compounds investigated were fully optimized at the AM1 level to obtain reliable molecular structures.

According to Figures 1 and 2, the flexible aspects of the side chain of the different compounds are governed by three (ϕ , θ , and ψ), two (ϕ and ψ), or only one (ϕ) torsional angles. Consequently, for those compounds that have only one torsional angle in their side chain, a grid scan around ϕ was carried out with a grid resolution of 15°. At each position a full geometric optimization, except for the angle under study, was performed. Compound **23** has two torsional angles in its side chain; therefore, the conformational study of this molecule was performed from a double scan of ϕ versus ψ .

For those compounds with three torsional angles in their side chains, a systematic conformational search was carried out to determine the low-energy conformations of these molecules.

The electronic study of the molecules was carried out using molecular electrostatic potentials (MEPs). These MEPs have been shown to provide reliable information, both on the interaction sites of molecules with point charges and on the comparative reactivities of these sites (Politzer and Truhlar,

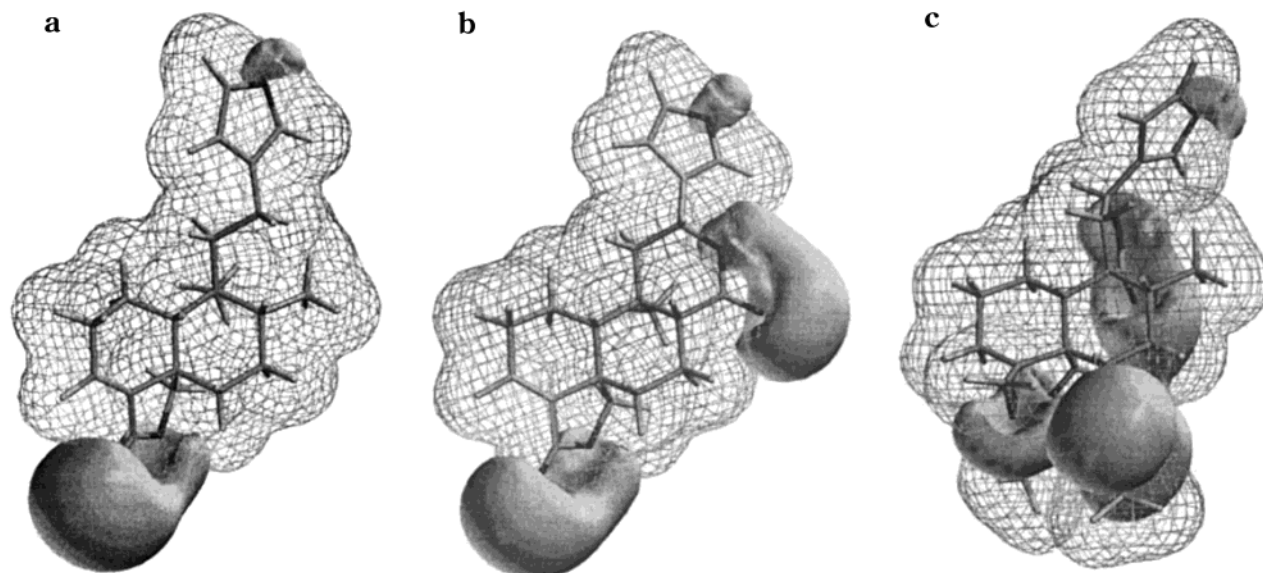


Figure 3. MEPs calculated using the AM1 optimized geometries: (a) compound **1**; (b) compound **8**; (c) compound **15**.

1981; Carrupt et al., 1991; Jáuregui et al., 1997). The MEPs were calculated using AM1 wavefunctions from the SPARTAN program (SPARTAN, 1995).

RESULTS AND DISCUSSION

Clerodane Diterpenoids. The feedant-deterrent activities obtained for the clerodanes shown in Figure 1 are summarized in Table 1. The results elucidated some general trends in structure–activity relationships. First, all of the active compounds have a furan ring in their side chain (compounds **1**, **2**, **5**, **8**, **9**, **15**, and **16**). These results suggest that the presence of a furan group in the side chain is mandatory for an acceptable anti-feedant effect in this series. However, the lack of activity obtained for compounds **6**, **7**, **10**, **12–14**, **18**, and **19** clearly indicates that the presence of a furan ring would be a structural requirement but not by itself sufficient for the anti-feedant activity.

In an attempt to find other potentially reactive sites for the ligands, we have evaluated the electronic aspects of the molecules.

Figure 3 gives the MEPs obtained for compounds **1**, **8**, and **15**. These results account for the general characteristics of the electronic behavior of clerodanes reported here. Computations have been carried out for the rest of the compounds of this series with these latter results being considered representative of the overall phenomenon.

The common salient feature of these maps is the existence of localized minima in the vicinity of the β -furyl group along with either the carbonyl α,β -unsaturated system or the disubstituted oxirane ring (compound **15**). These results led us to speculate that two binding sites would be necessary to produce the anti-feedant activity of these clerodanes. Comparison of the structures of **1**, **2**, **8**, and **9** with those of **5**, **15**, and **16** suggests that the disubstituted oxirane ring moiety may function as a bioisosteric replacement for the carbonyl α,β -unsaturated group.

To more clearly define the functional binding moieties of these compounds, we evaluated a series of clerodane analogues (Figure 1). SAR studies allow us to identify the structural features that are essential for anti-feedant activity. The first one relates to the substituent in the

side chain. Replacement of the furan ring with a butenolide ring (compound **3**), a tetrahydrofuran ring (**4** and **11**), or a mixture of diastomeric ketals (**17**) or the replacement of the hydroxylic chain with an *E* double bond in position 12 by an acetylated chain with an *E* double bond in position 13 (**20** and **21**) results in compounds devoid of anti-feedant activity.

Compound **3**, with a carbonyl α,β -unsaturated system in ring A, and compounds **17** and **21**, having a disubstituted oxirane ring, are inactive. It is interesting to note that these molecules also lack a furan ring in their side chains. Thus, the lack of activity of these compounds highlights the importance of an apparent synergistic effect of two active centers for this series.

In an attempt to verify this hypothesis, molecule **1** was chemically modified to obtain the derivative **7**. It was inactive, supporting the idea that at least two different sites are necessary to produce the anti-feedant activity.

The anti-feedant activity shown by **8**, **10**, and **12** provides additional support for this hypothesis. Whereas compound **8** was active, compounds **10** and **12** were inactive. It should be noted that the principal structural difference among these compounds is the loss of the α,β -unsaturated system for molecules **10** and **12**. The only structural difference between compounds **8** and **12** is the different position of the double bond in ring A. Whereas compound **8** was active, molecule **12** was inactive. These results emphasize the importance of the presence of a carbonyl α,β -unsaturated system, indicating that a carbonyl group alone is not sufficient to produce activity. The lack of activity of compound **10** illustrates this point well.

Particularly noteworthy was the lack of activity obtained for compounds **6** and **13**, even though they possess two potentially reactive centers. The presence of a carbonyl group in the side chain of **6** introduces profound electronic and conformational effects in the furan ring. These aspects, which could explain the lack of anti-feedant activity obtained for compound **6**, have been previously discussed in detail (Enriz et al., 1994a).

There are several explanations for the lack of activity obtained for **13**. One possibility is that this could be a manifestation of the lower reactivity of a more hindered

oxirane ring in **13** in comparison with the disubstituted oxirane ring of the active compounds **5**, **15**, and **16**. Another possible contributing factor could be the different spatial ordering for compound **13** with respect to that obtained for the active compounds.

Table 2 gives the interatomic distances obtained for the two potentially reactive centers of clerodanes. These distances were calculated from the low-energy conformations obtained for each molecule.

It is interesting to note that all of the active compounds have interatomic distances between the putative active centers in a range between 9.8 and 10.8 Å. Compounds **14** and **15**, having interatomic distances of ~6 Å, were inactive. From these results, we can suggest a new structural requirement for the antifeedant activity of these compounds, namely, that the optimal distance between the two potentially reactive centers has to be in a range between 9.8 and 10.8 Å.

It had been reported by several groups, including ours, that for the antifeedant effect of clerodanes the presence of at least two active centers is necessary. The results reported here are additional support for this hypothesis. Our results confirmed that in highly active compounds, a furan ring in the side chain and a carbonyl α,β -unsaturated (or spiroepoxide) group in ring A are essential.

Previously we had reported that it was not possible to find a statistically significant correlation between the log PFI values and the hydrophobicity constants obtained by HPLC-RP (log K_w), either alone or in conjunction with connectivity indices for these compounds (Luco et al., 1994). The present results confirm that the lipophilicity of clerodanes is much less important than are stereoelectronic factors in the production of the antifeedant response. Further studies concerning the quantitative relationship between physical properties and activity are in progress; preliminary results are in complete agreement with the results reported here.

Azadirachtin and Withanolides. Azadirachtin was chosen as a reference compound for comparative purposes. The feedant-deterrent activities obtained for azadirachtin **22** and withanolides **23–25** (Figure 2) are shown in Table 1. Under the same conditions, the antifeedant activity of **22** was comparable to those of the most active compounds of both clerodanes and withanolides.

The antifeedant activity level of compounds **23** and **24** is remarkable. This result is not surprising because compound **23** is a major component of *Nicandra physaloides* (Solanaceae), a reputed fly repellent of Peruvian origin. Aqueous extracts of leaves of this plant have been shown to inhibit the feeding of larvae of various insect species. From this plant other pesticides and antifeedants have been isolated (Begley et al., 1972). In contrast, molecule **25** was inactive. The lack of activity shown by compound **25** was somewhat surprising in view of the strong activities obtained for the other withanolides. Molecular modeling studies performed using these compounds are described in the next section and provide some explanation for the different activities observed.

The MEPs of withanolides were examined (Figure 4). It was possible to single out a common feature of the MEPs of these compounds, which was comparable to those obtained for the clerodanes, namely, the presence of sharply negative lobes in the MEP, close to the

Table 2. Measured Interatomic Distances between the Oxygen Atoms of the Potentially Reactive Groups in the Molecules from AM1 Geometry^a

| Compound | Heteroatom involved | Distance (Å) | |
|-------------|----------------------------------|------------------------------------|-------------|
| [1] | O ^f | O=C ¹⁸ | 9.96 |
| | | C ¹⁸ -O-C ¹⁹ | 11.25 |
| [2] | O ^f | O=C ¹⁸ | 9.81 |
| | | C ¹⁸ -O-C ¹⁹ | 11.71 |
| [5] | O ^f | C ⁴ -O-C ¹⁸ | 9.55 |
| [8] | O ^f | O=C ¹⁸ | 9.26 |
| | | C ¹⁸ -O-C ¹⁹ | 11.20 |
| [9] | O ^f | O=C ¹⁸ | 9.81 |
| | | C ¹⁸ -O-C ¹⁹ | 11.03 |
| [13] | O ^f | C ³ -O-C ⁴ | 6.66 |
| [15] | O ^f | C ⁴ -O-C ¹⁸ | 10.32 |
| [16] | O ^f | C ⁴ -O-C ¹⁸ | 10.35 |
| [22] | O ^G | TigO-C ¹ | 10.6 |
| | | AcO-C ³ | 8.9 |
| [23] | C ¹ O | O ^E | 10.4 |
| | | C ²⁴ -O-C ²⁵ | 9.9 |
| | | HO-C ²⁶ | 10.3 |
| | C ⁵ -O-C ⁶ | O ^E | 10.6 |
| | | C ²⁴ -O-C ²⁵ | 11.0 |
| | | HO-C ²⁶ | 11.3 |
| [24] | C ¹ O | O ^E | 8.9 |
| | | O=C ²⁶ | 11.0 |
| [25] | C ¹ O | O ^E | 6.4 |
| | | O=C ²⁶ | 6.3 |

^a Inactive compounds having interatomic distances on the order of 6.5 Å are shown in bold.

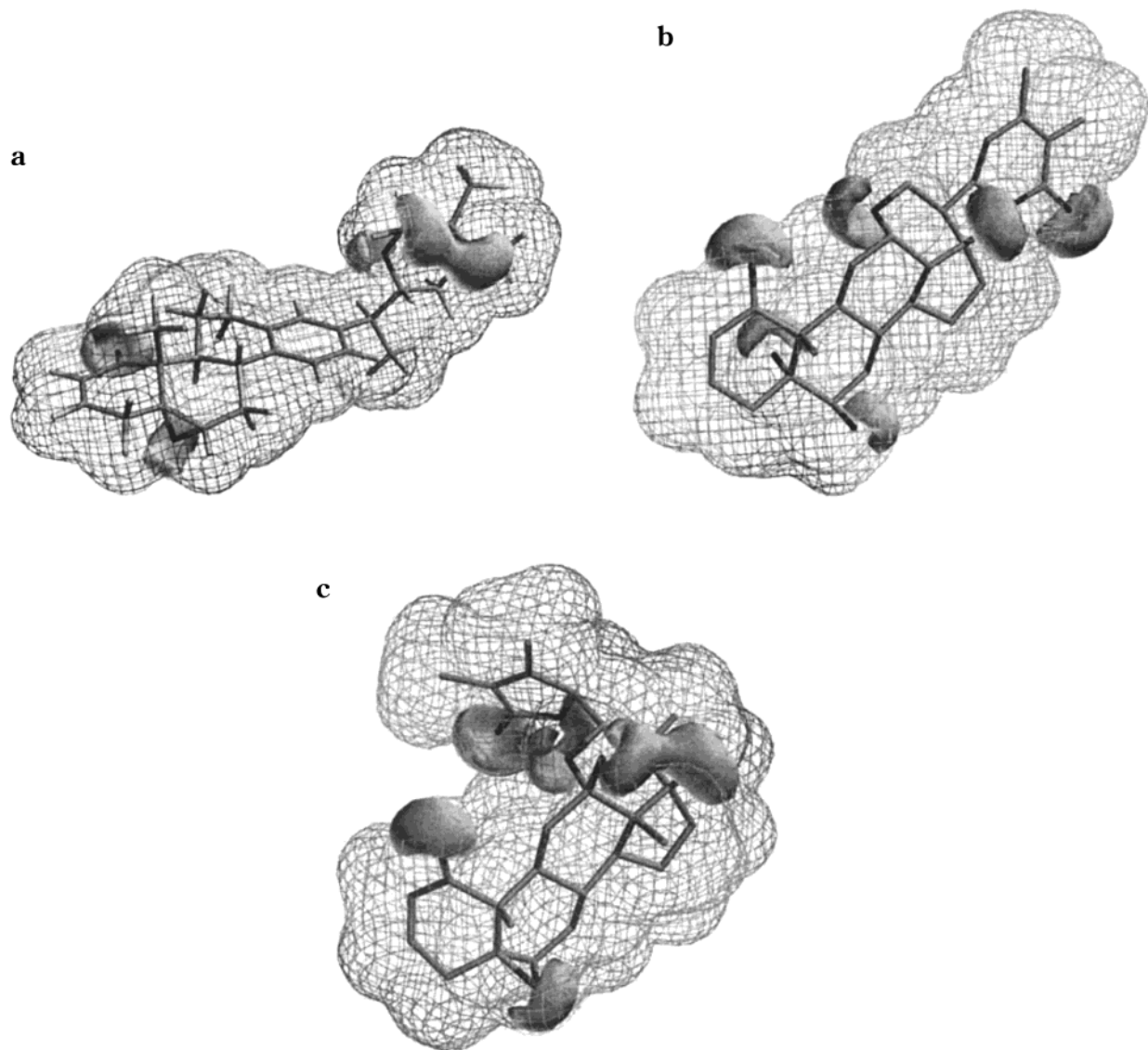


Figure 4. MEPs calculated using the AM1 optimized geometries: (a) compound **23**; (b) compound **24**; (c) compound **25**.

oxygenated groups in the A ring of the basic nucleus and those in the proximity of the heteroatoms of the side chain.

From Figures 3 and 4, we can graphically estimate that the MEPs of withanolides are remarkably similar to those of clerodanes. This assumption was supported by a qualitative comparison of the isopotential maps obtained through the semiempirical computer modeling of the substructures involved.

It is interesting to note that the interatomic distances between the potentially reactive centers of the active withanolides **23** and **24** are quite similar to those obtained for clerodanes (Table 2). In contrast, the interatomic distance between the potentially reactive centers of **25** was markedly lower with respect to the active compounds. Thus, it seems to be reasonable to assume that the lack of antifeedant activity of **25** is a consequence of the different spatial ordering of this compound.

Recently, a detailed conformational analysis of azadirachtin and derivatives has been reported (Baldoni et al., 1996, 1997, 1999). These papers report that the low-energy conformation of azadirachtin is the biologically relevant form of this compound. In the present paper we use this low-energy conformation to evaluate

the MEP of this compound (Figure 5). Here it is extremely difficult to decide which oxygen atoms mimic the oxygen in the furan ring of clerodanes and whether the other oxygen atom is simulating the carbonyl α,β -unsaturated system or the epoxide group. However, previous experimental and theoretical studies (Hansen et al., 1992; Baldoni et al., 1996, 1997) indicate that oxygen atoms at C₁, C₃, and C₂₀ seem to be involved in the molecular recognition process. These groups show the presence of sharply negative lobes in the MEP, which are comparable to those observed in the MEPs of the active clerodanes and withanolides. Particularly noteworthy was the fact that the interatomic distance between these putative active groups of azadirachtin is closely similar to those obtained for the potentially reactive centers of both clerodane and withanolide molecules (Table 2).

The hydrophobic aspects of these compounds were evaluated from the hydrophobicity constants (K_w) obtained by HPLC-RP. These results are shown in the caption of Figure 2. From the limited number of molecules studied here it is not possible to definitively describe a hydrophobic feature for these compounds. However, these results suggest that hydrophobicity does not directly correlate with an increase in feedant-

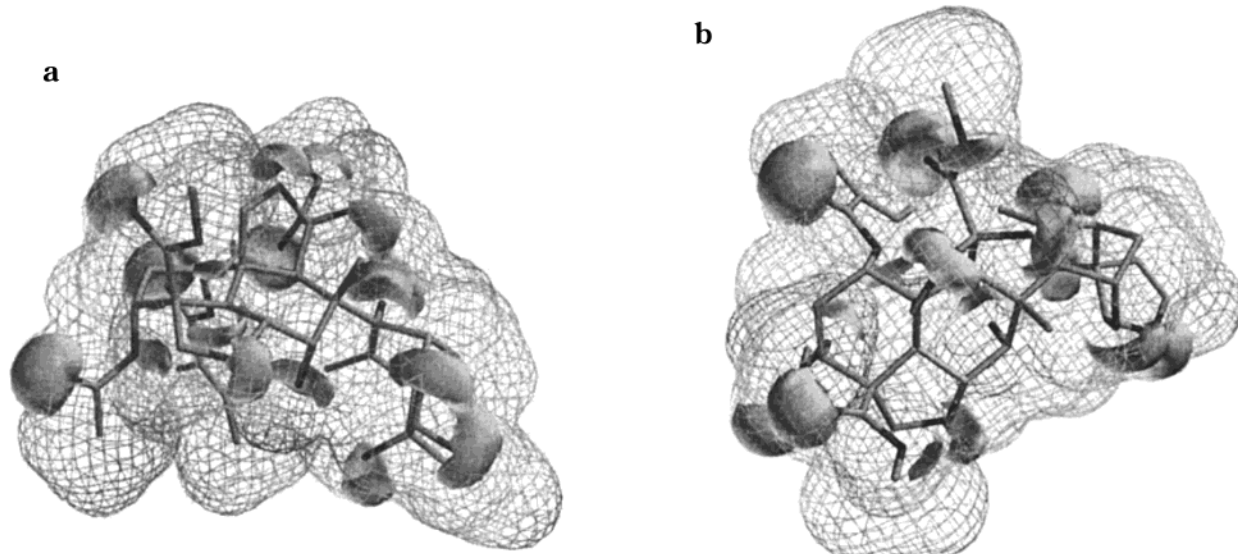


Figure 5. MEP obtained for azadirachtin **22** from AM1 calculations using the low-energy conformation reported in Baldoni (1996, 1999).

deterrent activity against *T. molitor*. Once again, it appears that the stereoelectronic factors of these compounds are more important than hydrophobic aspects in the production of antifeedant activity.

The similar spatial orderings and electronic distributions for the obtained azadirachtin, clerodanes, and withanolides suggest that these compounds interact at the biological receptor in a chemically similar manner. This is particularly apparent after examination of certain detailed stereochemical agreements.

Conformational Analysis. Conformational analyses were conducted on the compounds reported here as described under Computational Analysis.

Low-energy conformations of the compounds were obtained and compared. Conformers within 3 kcal/mol of the global energy minimum were retained. The other conformations were discarded.

For simplicity of presentation in this paper, clerodanes are divided into three structural classes: 1, compounds containing a flexible connecting chain (**1–7**); 2, conformationally restricted analogues containing a C₁₂–C₁₇ δ -lactone system (**8–12**); and 3, conformationally restricted analogues containing a C₁₂–C₂₀ spiro-lactone system (**13–19**).

Compounds **20** and **21** were inactive. Because they do not have a furan ring in their flexible chain, calculations were not performed for these compounds.

A systematic conformational search performed on compounds **1–5** and **7** indicates the existence of two minimum energy conformations within 3 kcal/mol of the global minimum. AM1 calculations predict the conformation with $\phi \cong 303^\circ$, $\psi \cong 179^\circ$, and $\theta \cong 17^\circ$ as the preferred form for these compounds. For compound **6** theoretical calculations predict the existence of six conformations, the conformation with $\phi = 285^\circ$, $\psi = 236^\circ$, and $\theta = 183^\circ$ being the global minimum.

Compounds **8–12** have a common structural feature; they have a C₁₂–C₁₇ δ -heterocyclic lactone (ring C, Table 1) fused with the *trans*-decalin moiety. The conformational behaviors observed for these compounds are similar and are shown in Figure 6.

Compounds **13–19** have a C₁₂–C₂₀ spiro-lactone system. This five-member heterocycle is perpendicular to the *trans*-decalin group. Whereas compounds **13** and

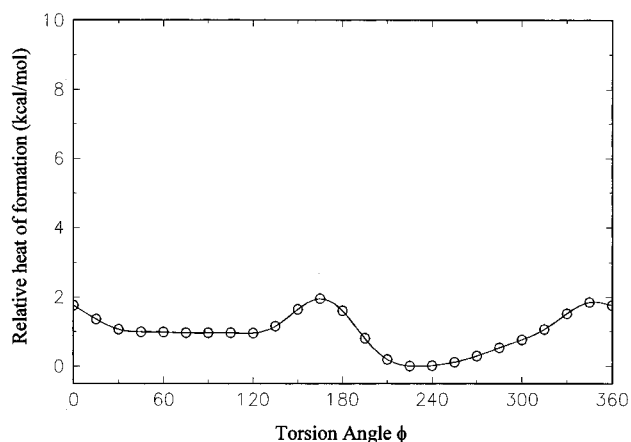


Figure 6. Potential energy curve obtained for the torsional angle ϕ of compound **8**. This curve is representative for compounds **9–12**.

14 have a 9*S* configuration, compounds **15–19** gave a 9*R* configuration. This different configuration is primarily responsible for the different spatial ordering obtained for these compounds. In molecules **13** and **14** the β -furyl group is displaced toward the A ring of *trans*-decalin. In contrast, for compounds **15–19** the β -furyl displacement is toward the B ring of the *trans*-decalin system. This different spatial ordering explains the lower interatomic distance between the potentially reactive groups obtained for compound **13** in comparison with molecules **15** and **16** (Table 2).

Figure 7 gives the potential energy surface (PES) obtained for compound **23** when the torsional angles ϕ versus ψ are varied every 15° . AM1 calculations predict the existence of six minimum-energy conformations within 0.5 kcal/mol of the global minimum and suggest in particular that the most stable form is that in which the ϕ and ψ angles are *g* (*gauche*, 295°) and *a* (*anti*, 167°).

The molecular superimposition of **24** and of **25** is shown in Figure 8. In this superimposition **24** has ring E axial at C₂₀, whereas compound **25** has this ring equatorial at C₂₁. It is clear that **25** cannot place ring E in the same region of space as the corresponding ring of **24**, and consequently **24** fits very badly.

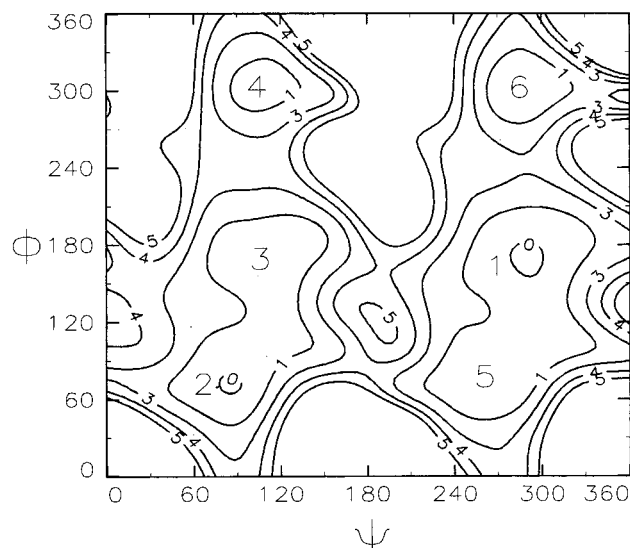


Figure 7. Potential energy surface (kcal/mol) obtained for compound **23** when the torsional angles ϕ versus ψ are varied each 15° . The six different low-energy conformations are denoted 1–6. The global minimum is shown with number 1.

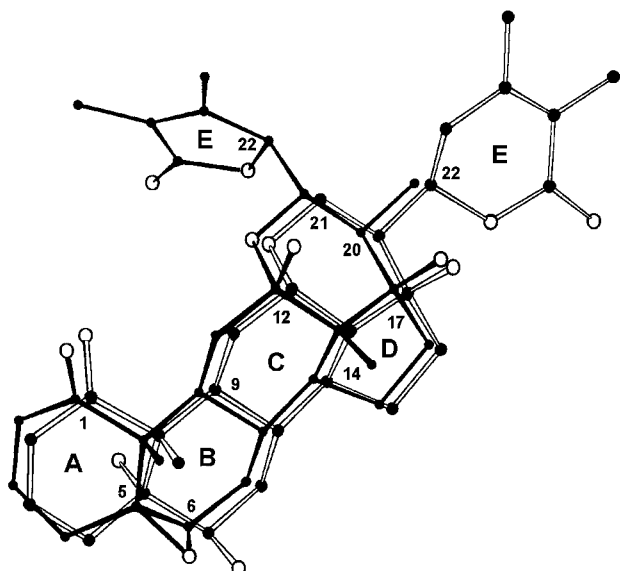


Figure 8. Superposition of the lowest energy conformers of compounds **24** and **25**.

The interatomic distances observed between the potentially reactive groups of clerodanes and withanolides are summarized in Table 2. These results indicate that the optimum interatomic distance between these groups for the production of antifeedant activity is ~ 10 Å. Compounds having lower interatomic distances were all inactive. Thus, it is reasonable to think that the antifeedant activity is strongly dependent on the distance between the apparent reactive centers.

CONCLUSIONS

We report here a group of clerodanes and withanolides acting as antifeedant agents. Among them compounds **8**, **9**, **15**, and **23** and some of their congeners exhibited remarkable feedant-deterrent activity against *T. molitor*.

SAR studies on the clerodane compounds including the use of MEPs and molecular modeling have allowed a model to be proposed for the recognition of the

minimal structural requirements for the production of the biological response by these compounds. Thus, a furan ring in the side chain and a carbonyl α,β -unsaturated (or *spiro*-epoxide) group appear to be indispensable. Also, our results indicate that the optimum interatomic distance between these moieties is ~ 10 Å.

An important feature in biological considerations is that the stereoelectronic factors of clerodanes are more important than their hydrophobic aspects as determinants of antifeedant activity. These results provide useful information for the determination of minimum structural requirements of the clerodane compounds, which can be the basis for the design of new molecules with desired properties.

Although the molecular mechanism of the antifeedant activity of the compounds reported here (azadirachtin, clerodanes, and withanolides) is unclear, the stereoelectronic complementarity observed among these molecules is noteworthy. Thus, on the basis of our results it is reasonable to imagine that a closely related chemical mechanism for these compounds produces the biological response. We are not attempting to generate a definitive and complete model in this paper; further work in this area including compounds of other series needs to be done. However, we believe our results may be helpful in obtaining a full understanding of the action mechanism of these compounds.

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