Structure–Activity Relationship of Clerodane Diterpenoids Acting as Antifeedant Agents

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Considering the feeding-deterrent activities exhibited toward *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) of several diterpenes with the clerodane skeleton isolated in our laboratory, a structure–activity relationship study was undertaken. A series of different clerodane diterpenoids was analyzed by performing an exhaustive study of their electronic and conformational behaviors. The results indicate that the antifeedant activity of these clerodane diterpenoids is mediated, at least, through two binding sites. Apparently one important factor contributing to the biological activity is a high conformational flexibility for the torsion angle of the β-furyl group.

Keywords: *SAR; insect antifeedant; clerodane diterpenoids; Tenebrio molitor (L.)*

INTRODUCTION

The need to protect our food supply from predatory attack using more ecologically acceptable methods has led to a rapidly growing interest in behavior-modifying chemicals from natural sources. Current environmental pressures and rapidly developing resistance to conventional insecticides provide the impetus to study new, more ecologically acceptable methods of pest control. 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.

Among the plant-derived chemicals, terpenoid compounds have often exhibited potent activity as insect antifeedants (El-Naggar et al., 1980; Rose et al., 1981; Picman, 1986; Bentley et al., 1988). In a previous paper, Simmonds et al. (1989) evaluated the antifeedant properties of 19 clerodane diterpenoids. The overall picture which emerges from these evaluations shows that small structural variations can produce drastic changes in the antifeedant activity of these compounds, making them particularly suitable for studies in structure–activity relationships. Considering different diterpenes having the clerodane skeleton isolated in our laboratory and the feeding-deterrent activities exhibited toward *Tenebrio molitor* larvae, a structure–activity relationship study was undertaken.

When only the structure of compounds characterized by a common biological activity is available and structural information on the receptor active site is lacking, the stereoelectronic requirements necessary to elicit the activity can be investigated through comparative analysis of the physicochemical properties of the ligands.

The present structure–activity study was carried out in an attempt to establish the structural requirements for the antifeedant effect of clerodane diterpenoids.

CALCULATIONAL METHODS

Conformational analysis and energy minimizations were carried out using the semiempirical AM1 method (Dewar et al., 1985), included in the MOPAC 6.0 package (Stewart, 1990). To obtain reliable molecular structures, complete optimizations of the geometrical parameters for the different conformations of compounds under study were made. The starting geometrical parameters (bond angles and distances) were taken from X-ray data of structurally related compounds (Savona et al., 1978; Wagner et al., 1978).

To complete the conformational study, molecular mechanics calculations were performed using a potential atom–atom empirical method designed by our group (Jáuregui et al., 1979; Giordano et al., 1992). This empirical method has been previously used in other biological systems with satisfactory results (Enriz et al., 1993, 1994).

Compounds **1**–**4**, **7**, **8**, and **10** (Table 1) present a flexible side chain; therefore, the conformation can be described by the three torsional angles ϕ_1, ϕ_2, and ϕ_3. In the conformational study, uniform scanning using molecular mechanics calculations was first carried out. In this way the conformations caused by the combinations of torsional angles of the different molecules were considered. The conformations obtained by molecular mechanics were then studied using AM1 quantum mechanic calculations.

To more accurately describe the conformations arising from the combination of torsional angles ϕ_1 and ϕ_2, we carried out a uniform scan of both angles using molecular mechanics calculations and keeping fixed ϕ_3 in the values previously obtained. On the other hand, we scan the torsional angle ϕ_3 keeping fixed ϕ_1 and ϕ_2 in the preferred conformations. These calculations were performed every 15° using the AM1 technique.

Compounds **5**, **6**, and **9** present only one torsional angle ϕ_3, which was evaluated as described above.

Molecular electrostatic potential maps (MEPs) (Scrocco and Tomasi, 1973; Politzer and Truhlar, 1981; Carrupt et al., 1991) can be a useful descriptor of the recognition process between the ligand and the molecule active site; thus, the MEP distribution for the lowest energy conformation of each compound was analyzed. These MEPs were calculated from AM1 semiempirical wave functions in the plane containing the *trans* decalin ring of the molecules.

EXPERIMENTAL PROCEDURES

Insect. *T. molitor* (L.) (Coleoptera: Tenebrionidae) larvae were obtained from Facultad de Ciencias Agrarias (UNC), and a stock culture was maintained on bran in plastic boxes at 24 ± 1 °C under 16/8 light/dark photoperiod.

Test Compounds. Diterpenes **1** and **2** were obtained from *Baccharis crispa* (Sprengel) as was described previously (Tonn et al., 1979; Tonn and Giordano, 1980). **3** was isolated from *B. triangularis* (Haumann) (Gianello and Giordano, 1989).

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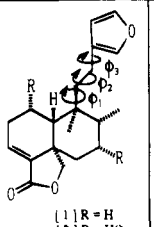
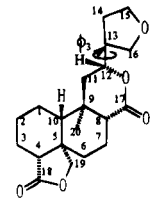
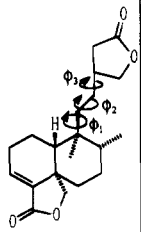
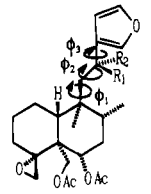
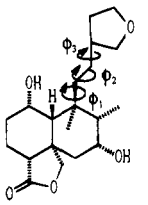
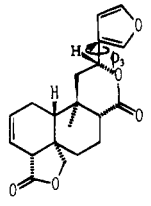
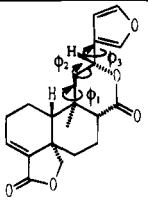
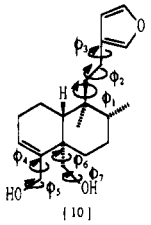
Table 1. List, Key Structural Features, and Antifeedant Activity of the Clerodane Diterpenoids[a]

MOLECULE	PFI ± SD (100ppm)	MOLECULE	PFI ± SD (100ppm)
 [1] R = H [2] R = HO	[1] 30.9 ± 4.8 a [2] 31.2 ± 4.1 a	 [6]	49.4 ± 2.8 c
 [3]	62.6 ± 3.8 c	 [7] R_1 = OAc; R_2 = H [8] R_1 = R_2 = O	[7] 31.4 ± 3.2a [8] 57.9 ± 5.0 c
 [4]	48.3 ± 6.6 c	 [9]	47.3 ± 4.7 c
 [5]	22.6 ± 4.6 b	 [10]	48.7 ± 5.8 c
ANOVA: a–b $p \leq 0.025$; a–c $p \leq 0.001$; b–c $p \leq 0.001$			

[a] The designation of the torsion angles in the connecting units is also included. (**1**) Hawtriwa lactone; (**2**) bacrispine; (**3**) entclerodan-3-ene-15,16:18,19-diolide; (**4**) hexahydrobacrispine; (**5**) bacchotricuneatin A; (**6**) hexahydrobacchotricuneatin A; (**7**) 6α,12S,19-triacetoxy-4α,18:15,16-diepoxyneoclerodan-13(16):14(15)diene; (**8**) 6α,19-diacetoxy-4α,18:15,16-diepoxy-12-ketoneoclerodan-13(16):14(15)-diene (**9**) salviarin; (**10**) kingidiol.

Compound **4** was prepared from **2** by catalytic hydrogenation (Tonn et al., 1979). From aerial parts of *B. rhetinoides* collected in Valle de las Leñas-Malargüe, Mendoza, Argentina (voucher Del Vitto-Petenatti no. 1367 UNSL), bacchotricuneatin A (**5**) was isolated (Wagner et al., 1978). Compound **6** was prepared from **5** by catalytic hydrogenation (Tonn et al., 1979). Compound **7** was isolated from *Teucrium grisebachii* (H. et A.) (Tonn et al., 1990). Diterpene **8** was obtained by selective hydrolysis of **7** (NaH/MeOH) followed by oxidation with Jones's reagent (Savona et al., 1984). Leaves of *Salvia reflexa* collected in Juana Koslay, San Luis, Argentina (voucher Del Vitto-Petenatti no. 6250 UNSL), were extracted with Me_2CO, and the resulting residue was percolated over silica gel using a solvent of increasing polarity (hexane/$CHCl_3$). After several purifications, compound **9** was obtained, which was identical in all respects to salviarin (Savona et al., 1978). Diol **10** was prepared from **1**: 30 mg of **1** in 30 mL of THF was boiled for 3 h under reflux with 60 mg of $LiAlH_4$; after the usual workup, 12 mg of kingidiol was obtained (Gianello et al., 1982; Bohlman et al., 1984).

Antifeedant Bioassay. Carrot slices (2.5 cm diameter and 0.5 cm thick) were coated with 100 μL/slice of test emulsions

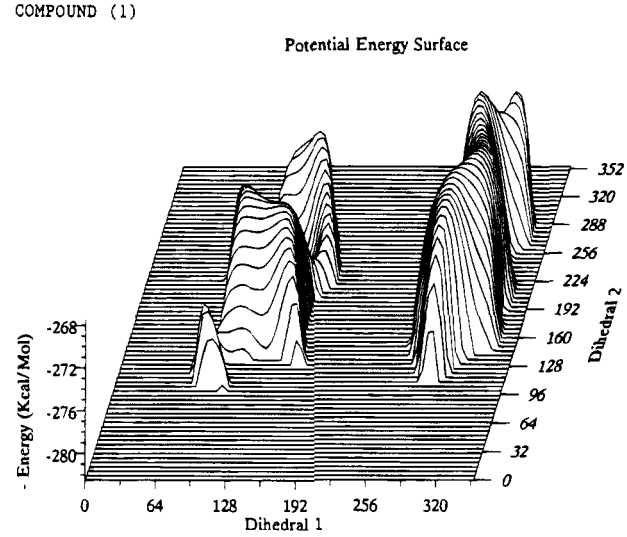


Figure 1. 3D plot of the conformational energy surface of compound **1** showing the position of the minima and the shape of the valleys. The maps were obtained using molecular mechanics calculations.

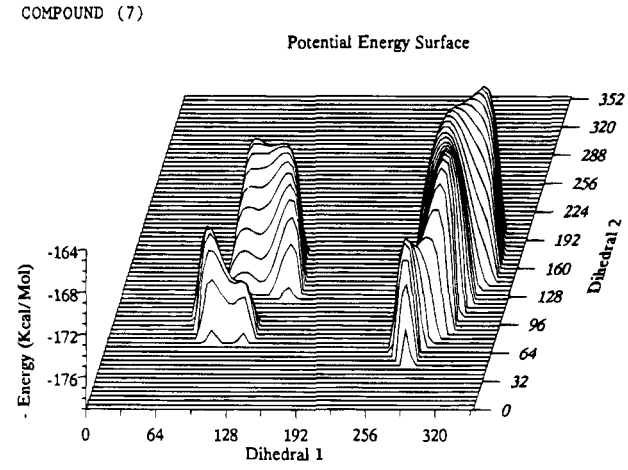


Figure 2. 3D plot of the conformational energy surface of compound **7**.

containing the target compounds. Emulsions were prepared at a concentration of 100 ppm in a mixture of H_2O/MeOH/Me_2CO (90:5:5) containing Triton CS-7 (0.1% by volume) 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 larvae in third instar 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 of the remaining slices from the initial weight of the appropriate test. This experiment was repeated eight times, in duplicate, for each of the compounds assayed. The activity was expressed as percentage of feeding inhibition (PFI) (Reed and Jacobson, 1983) according to

$$\text{PFI} = (\%\ \text{consumed of treated slices})/(\%\ \text{consumed of treated slices} + \%\ \text{consumed of untreated slices}) \times 100$$

A result of 50 indicates equal consumption of treated and untreated slices, while lower numbers 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.

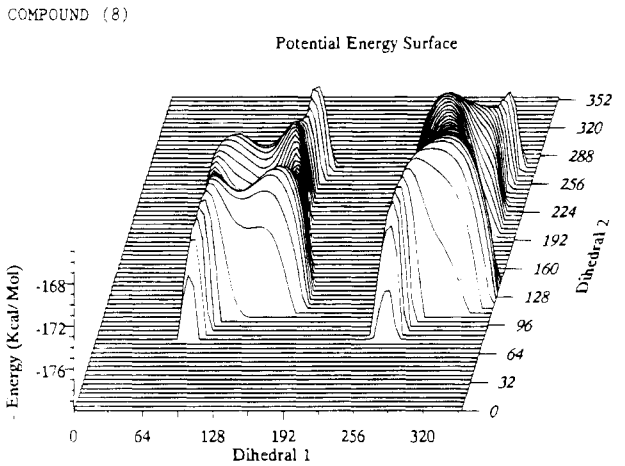


Figure 3. 3D plot of the conformational energy surface of compound **8**.

RESULTS AND DISCUSSION

The conformational energy maps from torsional angles ϕ_1 and ϕ_2 of compounds **1**, **7**, and **8** are shown in Figures 1, 2, and 3, respectively. The molecular mechanics calculations predict the existence of several minimum-energy conformations. The energy map contours present a similar topography. These maps are similar in shape; however, the map of compound **7** shows deep valleys with steep slopes. This different feature may be attributed to the bulky substitution of the acetate group of compound **7**. All of the conformations obtained are summarized in Table 2. It is interesting to note that molecular mechanics calculations are in total agreement with the AM1 results.

Our results indicate that the conformational behavior of molecules **3** and **4** is closely similar to that of molecule **1**. Energy profiles are ϕ_3 of molecules **1** and **5** showing the influence of β-furyl group orientation and the potential energy of the rotamers are given in Figure 4a,b. It is clear that the energy barriers for conversion between the different forms are small, and therefore the transition between different conformations appears to be a reasonable assumption. These results indicate that the rotation of the β-furyl group is poorly restricted. The energy profiles obtained for molecules **6** and **9** are closely related to that attained for compound **5**. It is interesting to note that compounds **5** and **9** differ only in the conformation of the A-ring; however, they have dramatically different activities. Our results suggest a closely related spatial ordering for both molecules with similar interatomic distances between the potentially

Table 2. Conformations, Torsional Angles, and Relative Energy of Conformers from Compounds 1, 5, 7, and 8 Using AM1 Calculations

molecule	conformation	torsional angle (deg) ϕ_1	ϕ_2	ϕ_3	rel energy (kcal/mol)
1	**1**–	303	179	1	0.00
	2–	300	174	91	0.03
	3–	111	182	261	2.15
	4–	110	182	77	2.27
	5–	140	267	110	5.40
	6–	150	254	277	5.92
5	**1**–	179	241	135	
7	**1**–	299	126	70	0.00
	2–	315	176	68	2.45
	3–	94	93	66	6.16
	4–	116	170	76	6.44
8	**1**–	285	236	183	0.00
	2–	311	115	179	0.58
	3–	104	101	177	2.62
	4–	129	262	184	3.30

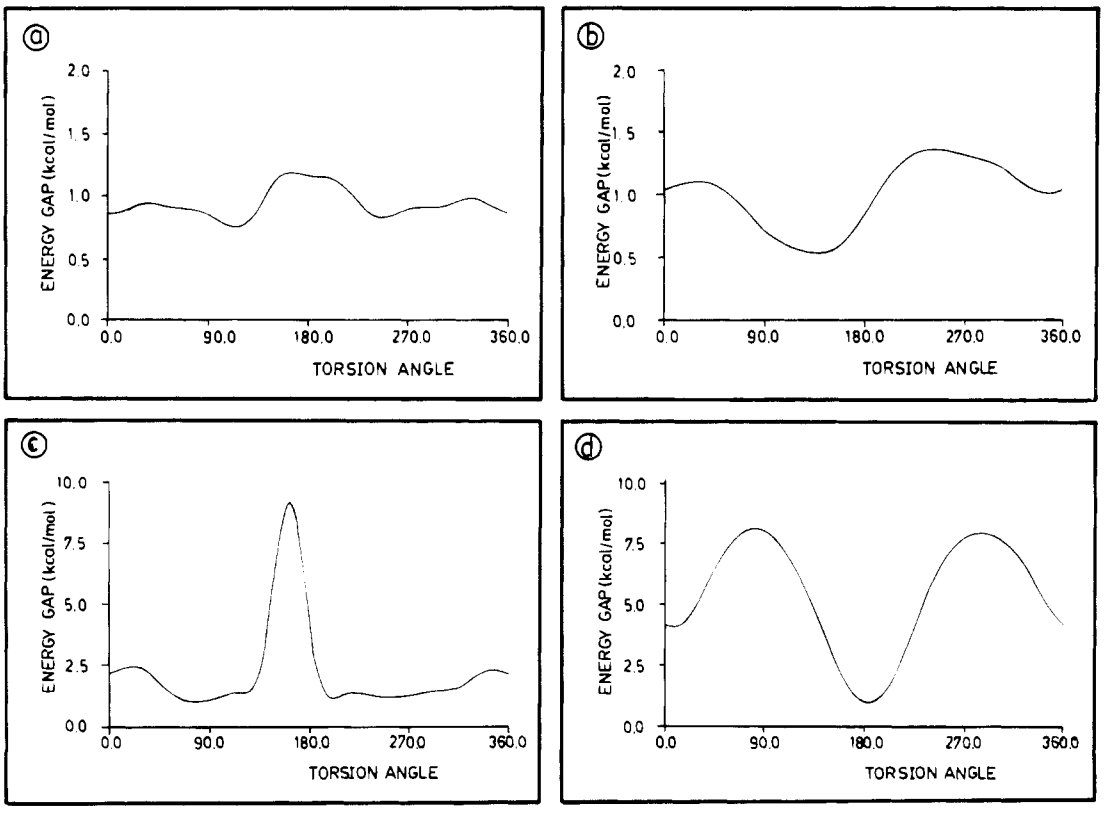


Figure 4. Energy gaps of the molecules from AM1 calculation showing the influence of β-furyl group orientation ϕ_3: (a) molecule **1**; (b) molecule **5**; (c) molecule **7**; (d) molecule **8**.

Table 3. Measured Interatomic Distances between the Potentially Active Groups in the Molecules from AM1 Geometry

	interatomic distances with respect to oxygen atom of β-furyl group (Å)		
molecule	C_{18} oxygen (carbonyl)	oxygen bridge (C_{18}–C_{19})	oxirane ring
1	9.96	11.25	
2	9.81	11.17	
5	9.26	11.20	
9	10.62	11.53	
7			9.55

active groups (Table 3). These results emphasize the importance of electronic effects rather than conformational factors in explaining the different antifeedant activity of these compounds. Thus, it is reasonable to think that the presence of an α,β-unsaturated double bond system on the A-ring could play a decisive role in the biological activity.

The curve obtained for **7** (Figure 4c) shows a maximum in 180° probable due to the acetate substituent; however, the rest of the curve is comparable with those obtained for the other molecules. In contrast, compound **8** (Figure 4d) has a very restricted conformational flexibility, wherein the C_{12} carbonyl group and the β-furyl group show a planar conformation as the highly preferred form. The energy differences between the most stable and other forms, as predicted by the AM1 calculations, are large enough to suggest that the transition between different forms is somewhat restricted.

It should be noted that while the inactive molecule **8** has a planar conformation highly preferred for compound **7**, which is active, this conformation is an energetically unfavored form. Applying the Restricted Analogue Approach strategy (Grunewald, 1979), we compare the conformation obtained for the flexible side chains of compounds **1**, **3**, **4**, **7**, and **8** with that attained for the conformationally restricted δ-lactone system of molecule **5**. The torsional angles ϕ_1 and ϕ_2 of molecule **5** are comparable to those obtained for the conformation 5 of compounds **1**, **3**, and **4**. These conformations show an energy gap of about 5 kcal/mol with respect to the global minimum; however, it must be pointed out that the preferred conformations of the flexible molecules (conformation 1 of Table 2) show a spatial ordering closely related to that obtained for compound **5** (Figure 5). These forms are comparable with conformation 2 of compounds **7** and **8**. AM1 calculations predict different molecular flexibilities for the above molecules. It is clear, however, that preferred conformations of these compounds show closely related spatial ordering with similar interatomic distances among potentially active groups.

Figure 6 shows the MEP maps obtained for compounds **1**, **5**, **7**, and **8**. The salient feature of these maps is the localized minimum in the vicinity of the β-furyl group and the α,β-unsaturated double bond or epoxide group.

Interesting to note is the different electronic distribution showed by molecule **8** as compared to the rest of the compounds. The different MEP map obtained for molecule **8** may be attributed to the conjugated π system introduced between the carbonyl group of C_{12} and the β-furyl group.

Observing the active compounds of Table 1, it is reasonable to think that the presence of a β-furyl group is necessary to produce the antifeedant effect. However,

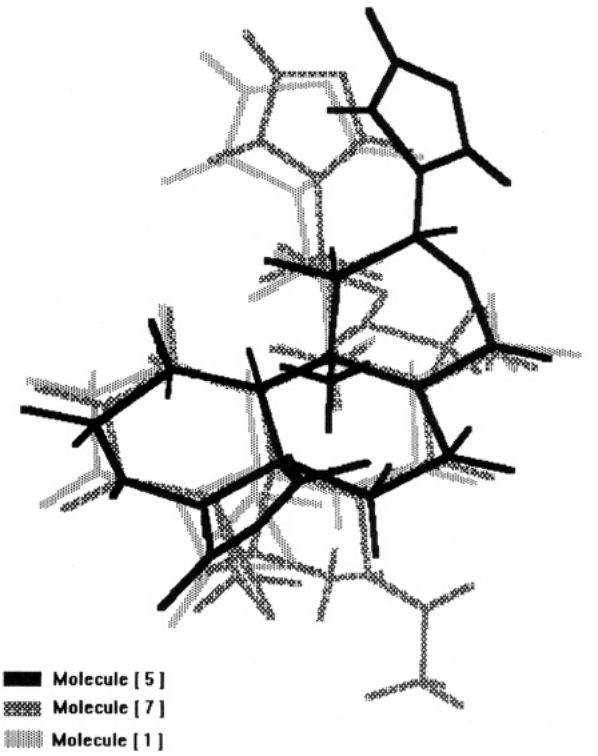


Figure 5. Stereoviews showing the superpositions of the low-energy conformation of compound **5** with conformation 1 of compound **1** and conformation 2 of compound **7**.

the question which arises is whether that structural requirement is the only one.

The MEPs obtained for the molecules show two potentially reactive sites: (i) a β-furyl group and (ii) an α,β-unsaturated double bond or oxirane ring.

It is interesting to note that the molecule **9**, which does not have the α,β-unsaturated system, is inactive.

On the basis of these results, we may speculate that two binding sites would be necessary to produce the antifeedant activity of these clerodane diterpenoids.

In an attempt to verify this hypothesis, molecule **1** was chemically modified to obtain the derivative **10** (see Experimental Procedures). It was inactive, supporting the idea that at least two different sites would be necessary to produce the antifeedant activity.

The conformational behavior of compound **10** is closely related to that obtained for molecule **1**; the possibilities of an intramolecular hydrogen bond in derivative **10** were taken into account. These results are summarized in Table 4.

Using the simple notion of receptor site occupancy, we may seek chemical features common to ligands to suggest chemical binding sites. We may assume that the β-furyl group of these compounds engages the receptor at a specific site, without disregarding the fact that the rest of the clerodane molecule can contribute to additional binding by interacting with some accessory region. Our results indicate that the two potentially active groups of the molecules are separated by a distance of about 10 Å (Table 3). The above hypothesis reporting two active sites in these compounds is in total agreement with previous reports (Simmonds et al., 1989; Belles et al., 1985; Bentley et al., 1990; Geuskens et al., 1983).

On the other hand, the conformational study suggests that a fairly free rotation for the β-furyl group could be important for biological activity to occur. This is

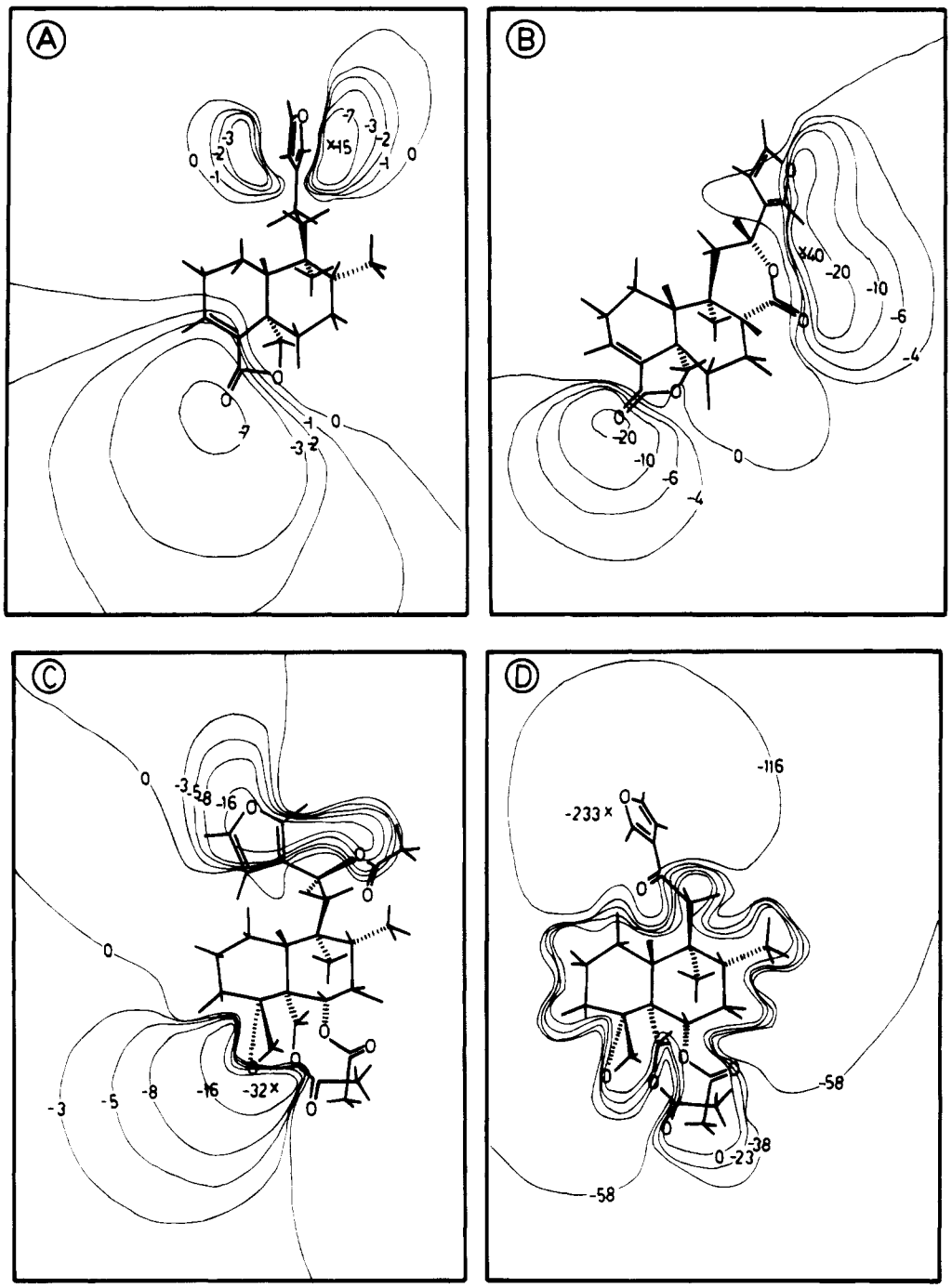


Figure 6. Molecular electrostatic potential maps of (A) molecule **1**, (B) molecule **5**, (C) molecule **7**, and (D) molecule **8** (values given in kcal/mol).

Table 4. Torsional Angles ϕ_4, ϕ_5, ϕ_6, and ϕ_7 of Compound 10[a]

torsional angle (deg)				
ϕ_4	ϕ_5	ϕ_6	ϕ_7	rel energy (kcal/mol)
287	62	350	212	0.00
40	53	349	178	0.30
56	308	350	174	0.97
287	266	347	161	2.00
280	38	177	185	16.93
44	297	177	190	17.48

[a] The torsional angles ϕ_1, ϕ_2, and ϕ_3 were kept fixed in the preferred conformation obtained for molecule **1** (ϕ_1 = 303, ϕ_2 = 179, ϕ_3 = 1); the calculations were performed from AM1 approach.

particularly apparent after examining certain detailed conformational differences; the derivative **8**, which contains the conformationally constrained β-furyl group, is inactive. The lack of activity of molecule **8** is consistent with the above idea.

In short, from the above results we can conclude that the presence of an α,β-unsaturated system, or one spiroepoxide substituent at C_4 in the clerodane structure, together with the β-furyl moiety at C_9, is important to evoke antifeedant activity. In addition, the free rotation of the β-furyl group could play a significant role in the biological activity.

The failure to find a statistically significant correlation between the log(PFI) (percentege of feeding inhibition) values and the hydrophobicity constants obtained by HPLC-RP (log K_w') alone or in conjunction with connectivity indexes for these compounds (Luco et al., 1994) indicates that the conformational and electronic

effects appear to be more important as determinants of antifeedant activity.

From the limited amount of molecules studied here it is not possible to conclude a definitive structural feature of these clerodane diterpenes acting as antifeedant agents. However, we believe our results may be helpful in the structural identification and understanding of the minimum structural requirements for these molecules and can provide a guide in the design of compounds with this biological activity. Further work in this area needs to be done to obtain a full understanding of the mechanism of action of these compounds.

ACKNOWLEDGMENT

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