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Insect Antifeedant Activity of Tetranortriterpenoids from the Rutales. A Perusal of Structural Relations

G. SURESH,*,† GEETHA GOPALAKRISHNAN,† S. DANIEL WESLEY,†
N. D. PRADEEP SINGH,† R. MALATHI,‡ AND S. S. RAJAN‡

Centre for Natural Products, SPIC Science Foundation, 64, Mount Road, Guindy, Chennai 600 032, India, and Department of Crystallography and Biophysics, University of Madras, Guindy Campus, Chennai 600 025, India

Structure-related insect antifeedant relationship of 56 limonoids (both natural and modified) from the plants belonging to the order Rutales was attempted considering substitution patterns, oxidation states, and hydrophobicity, as well as distant geometry derived through conformational analysis on molecular modeling. Orientation of the furan and hydroxylation at specific carbon sites have been shown to influence the antifeedancy against the fall armyworm, *Spodoptera litura*.

KEYWORDS: Antifeedant activity; limonoids; Rutales; structure−activity relationship; oxidation state; hydrophobicity

INTRODUCTION

After the isolation of azadirachtin from the neem seed kernels as an effective insect antifeedant against the desert locust (*1*), a number of limonoids from the order Rutales were screened for insect antifeedant activity, the results of which changed the then-prevailing belief that "limonoids seem to be remarkably bereft of physiological properties; we have examined many without finding anything beyond the characteristic bitter taste...."- (*2*). Champagne et al. (*3*) attempted to relate the structure of a number of limonoids with insect antifeedant activity and insect-growth regulatory (IGR) effects and indicated that the study has inherent difficulties with respect to drawing meaningful conclusions. This has been explained as, in part, due to interspecific differences among the bioassay organisms, intra-species differences in terms of insect growth stages used, diversity of bioassay systems employed, and variations in the modes of applications of limonoids (*3*, *4*). Despite the difficulties, it was concluded that the most active among the limonoids are the C-ring modified limonoids of the azadirachtin type followed by the intact apo-euphol types having a 14,15-epoxide and either a 19/28 lactol bridge or a cyclohexenone A ring.

Much of the literature concerning antifeedant activity of C-seco limonoids is limited to azadirachtins and salannin. Studying the antifeedant activity against fall armyworm, of a number of synthetic modifications of azadirachtin, Ley et al. (*5*) concluded that the hydroxyfuranacetal moiety is important for high levels of antifeedant activity, and information on precise spatial and electrostatic requirements of all the various oxygen substituents is also needed to understand the mechanisms of antifeedancy. Govindachari et al. (*6*), based on observations of the antifeedant activity of natural congeners of azadirachtin-A, showed that substitutions in the Decalin ring system, especially at C1, C3, C11, C12, and C29, can also influence antifeedant activity. Salannin, another C-seco limonoid, was also studied in detail for antifeedant activity against a variety of insect species (*4*, *7*−*9*) and was found to be as effective as azadirachtin-A at higher concentrations. Antifeedant activity of salannin and 14 synthetic derivatives against *Leptinotarsa decemlineata* showed that hydrogenation of the furan ring, replacement of the acetoxyl group, modification of the tigloyl group, and saponification of the methyl ester increased the activity multifold (*10*). A number of seco limonoids, such as nimbin, salannin, and gedunin, have been shown to be photolabile and the photomodifications at the furan and tigloyl groups in azadirachtins have been shown to affect antifeedant activity (*11*−*17*).

Antifeedant activity studies on protolimonoids and intact apoeuphol limonoids (*18*, *19*), indicated that they are active among the tetranortriterpenoids, next only to the C-seco compounds. Work concerning structure-related antifeedant activity of intact limonoids is restricted to cedrelone, four of its synthetic derivatives, azadiradione, and epoxyazadiradione (*18*). The antifeedant activities of these compounds were correlated to changes in hydrophilic sites of the molecules and to the presence of cyclohexenone in ring A.

A perusal of the structural diversity of limonoids clearly illustrates the specialization of the basic skeleton through oxidations, ring-opening and rearrangements, and cyclizations (*20*). The resultant changes in substitution patterns, oxidation state, hydrophobicity, molecular connectivity, electrostatic potential, conformation, and distance geometry are hence predicted to influence antifeedant activity. Although the molecular basis for action of antifeedants in insect gustatory systems is not known, experiments with established direct antagonists of major neuroreceptors indicated that antifeedant activity of insects has a strong association with $GABA_A$/glycine-

* To whom correspondence should be addressed. Phone: 91 044 235 1903. Fax: 91 044 235 1504. E-mail: sureshgovind55@hotmail.com.
† Centre for Natural Products.
‡ University of Madras.

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type amino acid receptors. On the basis of molecular modeling, common binding features for high antifeedant activity among polycyclic terpenoids were identified (*21*) which included an epoxide, π bonding sites separated by 5−6 Å, one or more electronegative oxygen centers, and polyoxygenation to maintain sufficient polarity.

The present investigation has attempted to consider skeletal specializations, oxidation states, molecular connectivity, and molecular modeling through overlap diagrams in order to understand antifeedant activity of limonoids to derive meaningful structure−antifeedant activity relationships.

MATERIALS AND METHODS

Insect Antifeedant Activity. *Spodoptera litura* L. (= *Prodenia litura* (F.) auctt.) (Noctuidae: Lepidoptera), a polyphagous pest of cotton, rice, tomato, tobacco, groundnut, castor, and legumes was used as a test insect for antifeedant studies. Field-collected larvae were cultured on castor leaves (*Ricinus communis* L.) in the laboratory at 25 ± 2 °C. Second-generation larvae (3rd instar) from the laboratory cultures were used for antifeedant bioassay. Dual-choice antifeedant bioassay was performed (*18*). ANOVA Neumann−Keul means of area fed in treated and control leaves were calculated using a ΔT leaf area measurement meter. Percentage feeding index (PFI) (*22*) was calculated using the formula

$$\text{PFI} = \left[\frac{\text{mean area (mm}^2\text{) fed in treated}}{\text{mean area (mm}^2\text{) fed in treated} + \text{mean area (mm}^2\text{) fed in control}}\right] \times 100$$

Skeletal Specialization (*S*) Values of Limonoids. Skeletal specialization of a limonoid (per carbon) (*S*) with respect to a precursor compound was calculated by the method of Das et al. (*20*). The number of bonds broken (connected to C) in the ring and the number of bonds (to C or O if this involves formation of a new ring) for each carbon of the limonoid were summed up, and the counts thus obtained were divided by the number of carbon atoms in the compound, giving the value *S*.

This is illustrated by the formula

$$S = \frac{\sum (b + f)}{n}$$

where *S* indicates the skeletal specialization, *b* is the number of carbon with bonds broken, *f* is carbon with new bonds formed, and *n* is the number of carbons in the compound.

Oxidation State (*O*) of Limonoids. Oxidation state (*O*) values of limonoids are determined (*20*) by counting, for each carbon of the compound, −1 for each bond to H and +1 for each bond to a heteroatom; the sum of these counts is divided by the number of carbon atoms of the compound. The loss of groups is considered to operate through oxidized intermediates, and for each of the broken C−C bonds (which results in the loss of a molecular moiety compared to that of the precursor), 3 points are added to the count.

This is illustrated by the formula

$$O = \frac{\sum (o + 3C - h)}{n}$$

where *O* is the oxidation state; *o* is the number of C−O bonds; *C* is the number of C−C bonds; *h* is the number of C−H bonds, and *n* is the number of carbons in the compound.

Chromatographic Hydrophobicity Constant (K'w). Chromatographic hydrophobicity constant (K'w) of limonoids was calculated by modifying the method of Luco et al. (*22*). In the present method K'w was calculated by studying the behavior of limonoids and their photoproducts in an analytical HPLC system (Shimadzu LC 8A) fitted with Merck RP_{18} (25 cm × 4.6 mm i.d., 5 μm) column. Acetonitrile/H_2O (35:65) was used as the solvent system at 1 mL/min and detected at 215 nm. Capacity factor (k'ϕ) was calculated using the formula

$$\text{k}'\phi = \frac{t_{R'}}{t_m}$$

where $t_{R'}$ is retention time of the limonoid/photoproduct; t_m is the retention time of the solvent in which the compound was dissolved (methanol). The log of capacity factor (log k'ϕ) was then plotted against ϕ_{CH_3CN} from which the slope (*S*) was calculated.

The hydrophobic constant of the given compound was then calculated using the formula

$$\log \text{k}'\phi = \log \text{K}'_w - S\,\phi_{CH_3CN}$$

where log K'$_w$ is the hydrophobic constant.

Molecular Modeling. The models of the limonoids were built using the software builder incorporated in Insight II loaded on an Octane silicon graphics work station. The built models were brought to a minimized energy conformation using CFF91 (consistent force field) force field (*23*, *24*) incorporated in the software Discover.

The total potential energy E_T of the model is given by

$$E_T = V_b + V_\theta + V_\tau + V_{nb} + V_{es} + V_{hb}$$

where V_b is bond energy, V_θ is bond angle energy, V_τ is torsional energy, V_{nb} is nonbonded energy, V_{es} is electrostatic energy, and V_{hb} is hydrogen bond energy.

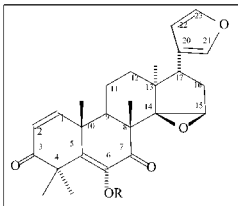
The bond lengths, bond angles, and torsional angles were obtained for the minimized models using the modules available in Insight II.

RESULTS AND DISCUSSION

Over 300 tetranortriterpenoids of the limonoid type have been isolated from Rutaceae and Meliaceae, and only a select few have been studied for their antifeedant activity. An earlier attempt to decipher structural features necessary for insect antifeedant activity of these limonoids based on published literature indicated that such an exercise was fraught with difficulties of interpretation due to interspecific differences among bioassay insects used, differences in growth stages of insects, and the variable methodologies employed.

To overcome these difficulties and to derive meaningful conclusions to understand the structural features necessary for antifeedant activity, we utilized a short-term bioassay using *Spodoptera litura* as the test organism utilizing 56 natural and modified limonoids (**Figure 1**) isolated and modified in our laboratory during the past decade. Antifeedant activities of natural and modified limonoids (**Table 1**) were assayed using circular leaf disk short-term bioassay with 3rd instar larvae of *S. litura* as the test organism, and percent feeding indices were calculated and presented (*4*, *6*, *18*). The chosen limonoids can be grouped into the following categories (a) intact apoeuphol limonoids, wherein all the four rings are intact with a furan attached to C-17; (b) C-seco limonoids and the related azadirachtins; (c) B,D-seco limonoids; and (d) D-seco limonoids. The modified limonoids were derived through photooxidation/microwave reactions(*25*−*27*).

Compounds **2**−**9** were the product of synthetic modifications of Cedrelone (**1**). All the naturally occurring limonoids utilized in the present study have intact furan attached to C-17 of the D-ring (except the azadirachtin type). Photooxidation of the naturally occurring intact limonoids resulted in the addition of an hydroxyl at C-23 and a carbonyl at C-21 in the intact furan. Additionally, a photoproduct with an epoxide between C-22 and C-23 in the case of nimonol and cedrelone was also observed. In the case of seco limonoids with intact furan, products with either hydroxyl at C-23 and carbonyl at C-21 or hydroxyl at



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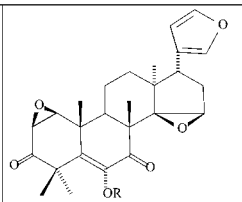

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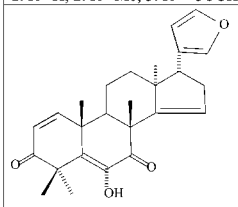

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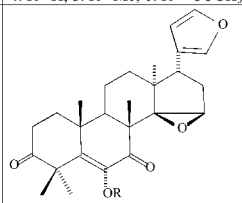

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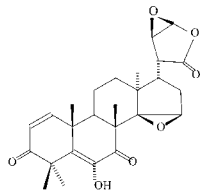

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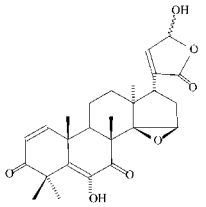

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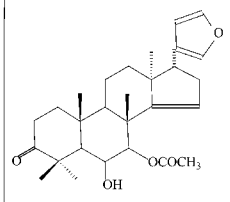

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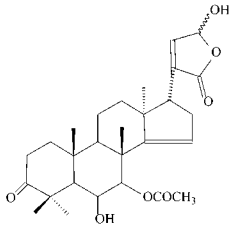

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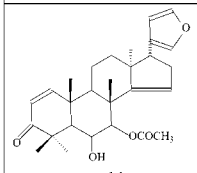

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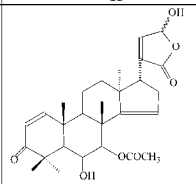

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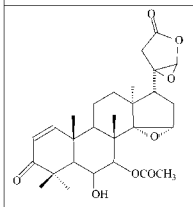

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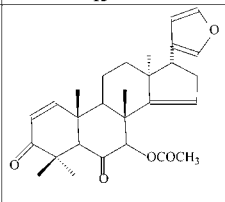

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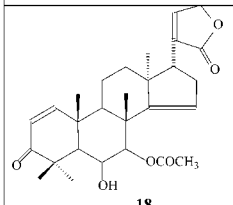

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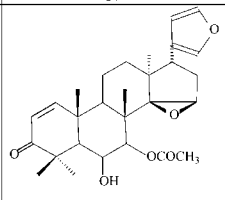

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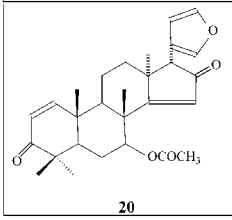

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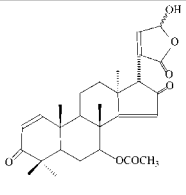

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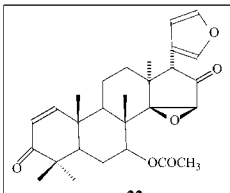

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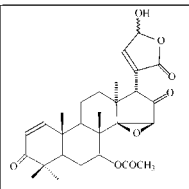

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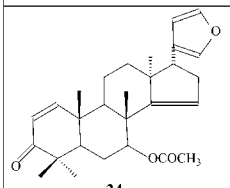

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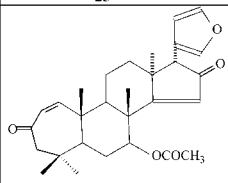

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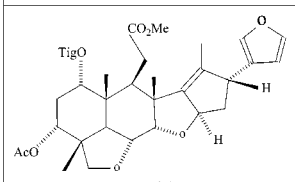

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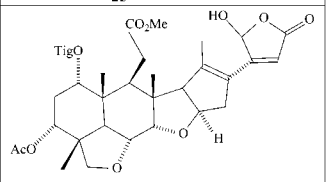

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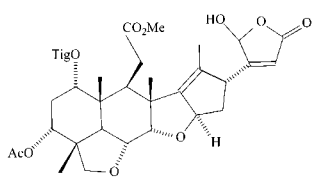

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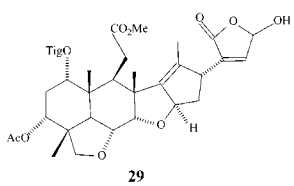

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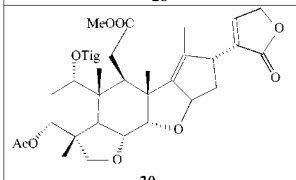

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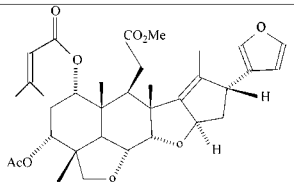

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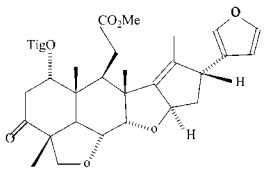

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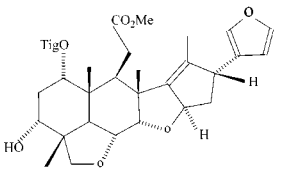

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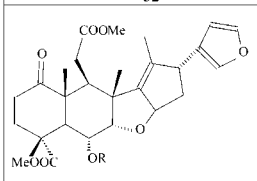
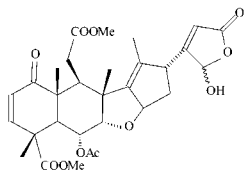


34: R= Ac; **38:** R=H



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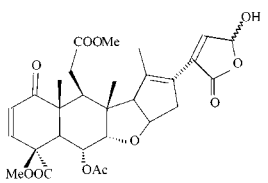

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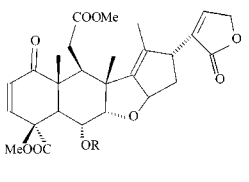

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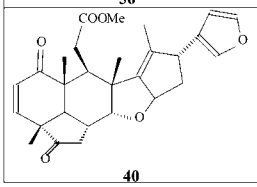

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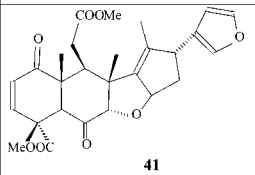

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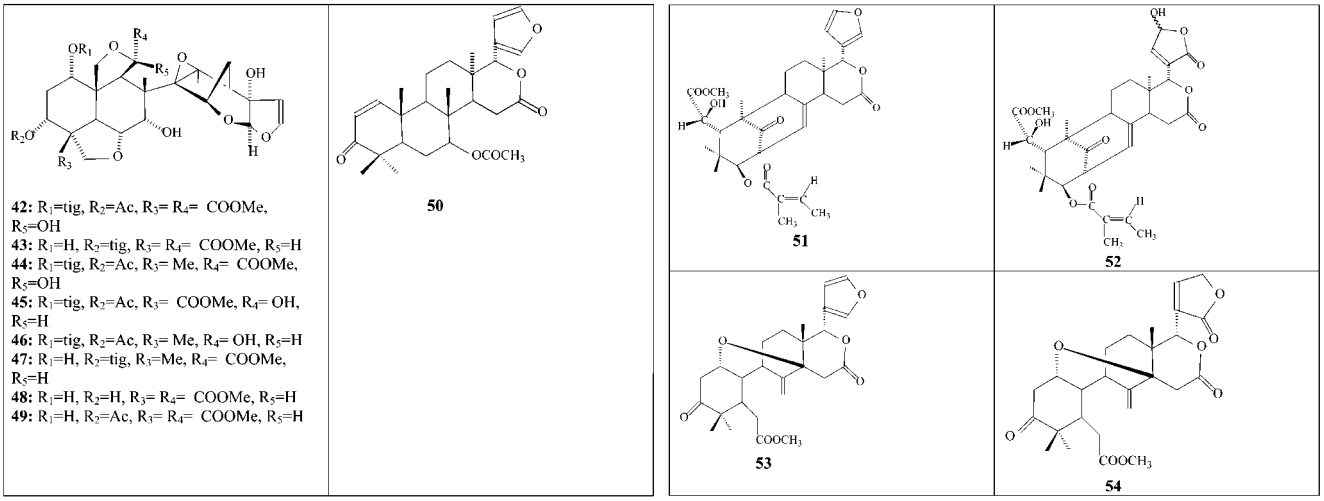


Figure 1. Structures of Natural and Modified Limonoids

C-21 and carbonyl at C-23 (isomer) were observed due to photoreaction. In the case of salannin an additional photoproduct with a shift of a double bond in the D-ring was also formed (**27**).

Among the natural limonoids tested, azadirachtin-A and its congeners were the most active antifeedants, followed by C-seco limonoids such as nimbin, 6-desacetylnimbin, and salannin (C-seco), and intact limonoids such as 14,15- epoxynimonol and azadiradione.

All the photomodified products of intact and seco limonoids with intact furan showed a marked increase in antifeedant activity compared to that of their substrates, indicating the oxygenation of intact furan will influence antifeedant activity. A comparison of the oxidation state (*O*) of individual limonoids (with intact furan) with that of their respective photoproducts showed oxygenation of furan resulting in higher antifeedant activity. But, linear regression analysis of the oxidation state of all the limonoids as a function of antifeedant activity showed that there is no positive correlation.

The activity profile of natural and modified limonoids in this study may also be looked at from the oxygenation point of view resulting in an epoxide, or a −OH or a free carbonyl in the furan ring. Desepoxycedrelone (**7**) will be an ideal starting point with Cederelone having a single epoxide at C14−15, while compounds **4**−**6** have an additional epoxide at C1−C2, compound **10** has one at C22−23, and the photoproduct of nimonol **16** having one at C20−21. Among the aforesaid compounds, only compounds **10** and **16** show appreciable increase in antifeedant activity compared to that of the parent compounds. This clearly illustrates the importance of oxygenation of the furan moiety as critical in increasing antifeedant activity. This can be further confirmed by the marked increase in antifeedant activity of intact limonoids **11**, **13**, **15**, and **23**, wherein an −OH and a carbonyl function are introduced at C-23 and C-21, respectively. In the case of photomodified C-seco limonoids (**29** and **36**), the increase in activity is not very appreciable, as the parent compounds are also active. The only exception to this observation is the lack of any appreciable increase in antifeedant activity of the photoproduct of azadiradione (**21**). This can be attributed to the possibility of formation of hydrogen bonding between the −OH group at C-23 and carbonyl function at C-16 of D-ring which are in close proximity, resulting in the nonavailability of the free hydroxyl for binding to the possible target site. The occurrence of doubling of carbon signals for C-16 in the D-ring clearly indicates such hydrogen bonding between the OH and the carbonyl of C-16 (*25*).

Addition of only a carbonyl function alone, without a hydroxyl in the furan moiety (**18**, **30**, **37**, and **39**), did not increase antifeedant activity, and in select cases, did reduce the activity appreciably. Compound **54** alone showed appreciable increase in activity compared to that of the parent compound. This further confirms the need to have a reactive −OH group in the furan ring at C-23 for maximizing the activity potential of the limonoids.

Among the substitutions in the Decalin ring system, C-3 substitution in C-seco compounds was shown to be critical in moult inhibitory activity (*28*) and antifeedant activity (*6*). Substitution of an hydroxyl at C-3 as a reactive species may also possibly indicate a second active site in limonoids for binding to the antifeedant receptors in insect mouth parts. A perusal of the intact limonoids in the present study show that the C-3 substitution is either a free carbonyl or an α,*β*- enone, and except in compound **33**, all other C-seco limonoids of the salannin and nimbin class do not possess a hydroxyl at C-3. As the compound **33** did not have a free hydroxyl at C-23, it was anticipated not to have high antifeedant activity. 3-tigloyl meliacarpin (**47**) (having an azadirachtin-like skeleton), possesses a C-1 hydroxyl and also showed higher antifeedant activity compared to that of all other natural azadirachtins (**42**−**46**). Azadirachtol (**48**) has a dihydroxy A-ring (at C-1 and C-3) and showed the maximum antifeedant activity among all the limonoids tested in the present study. This further indicates that a free hydroxyl in C-3 or C-1 would be the second active site in the binding to receptor sites.

Desacetylsalannin (**33**), a C-ring modified limonoid having a C-3 hydroxyl, was only half as active as azadirachtin-A, and hence was the candidate compound of choice to reconfirm the above observation. Photomodification of **33** resulted in desacetylsalanolide. Introduction of the hydroxyl in the furan ring increased antifeedant activity, indicating clearly two active sites in the triterpenoid molecule.

Quantitative structure−activity relationships studies predict behavior of molecules on the basis of detailed analysis of activity profiles of previously tested similar molecules. This approach takes into account receptor-related chemical interactions. In biological applications, it is predicted, the effects are usually due to noncovalent interactions which are conformation related (*29*) that include steric and electrostatic forces as well as distance geometry. In the present QSAR study, select factors such as furan orientation and distance geometry based on predicted activity sites on the molecule were considered.

With intact limonoids, oxygenation through photomodification resulted in consistent decrease in dihedral angle compared to

Table 1. Correlation of Insect Antifeedant Activity (expressed as PFI) of Limonoids to Structural Features[a]

	limonoid	PFI	log K'w	*O*	*S*	OF	dist. C-3/C-23
1	cedrelone[18]	51.5 (2.9)	1.2906	–0.77	0.08	167.94	10.84
2	cedrelonemethyl ether[18]	56.0 (6.6)	1.2602	–0.70	0.08	166.32	10.89
3	cedrelone acetate[18]	55.0 (7.5)	1.2714	–0.84	0.08	166.45	10.81
4	cedrelone epoxide[18]	43.5 (5.0)	1.3232	–0.73	0.15	168.09	10.91
5	methyl ether cedreloneepoxide[18]	54.0 (9.5)	1.3163	–0.66	0.15	166.48	10.97
6	acetate of cedreloneepoxide[18]	53.2 (9.0)	1.3072	–0.80	0.00	166.72	10.89
7	desepoxycedrelone[18]	55.3 (4.4)	1.2732	–0.84	0.08	150.27	11.11
8	dihydrocedrelone[18]	50.5 (6.3)	1.2856	–0.84	0.08	168.33	11.02
9	methyl ether of dihydrocedrelone[18]	56.5 (10.6)	1.2432	–0.85	0.08	166.92	11.06
10	cedrelone-22-epoxide	44.2 (2.8)	1.3328	–0.65	0.15	175.42	10.75
11	cedrelone pp-OH	22.4 (3.1)	1.3256	–0.65	0.08	149.88	10.89
12	isomeldenin	60.6 (5.9)	1.3149	–1.10	0.00	157.46	11.38
13	isomeldenin pp	28.3 (5.1)	1.3696	–0.99	0.00	136.38	11.59
14	nimonol	47.3 (4.1)	1.2303	–1.04	0.00	157.06	11.37
15	nimonol pp1	21.3(1.6)	1.3662	–0.93	0.00	136.41	11.59
16	nimonol pp2	15.8 (1.9)	1.2664	–0.93	0.00	136.41	11.63
17	6-oxonimonol	44.2 (2.8)	1.2018	–1.04	0.00	156.62	11.25
18	nimonol MW	44.2 (6.6)	1.2572	–1.00	0.00	158.10	11.62
19	epoxy nimonol	32.0 (3.9)	1.2436	–0.97	0.08	146.39	11.54
20	azadiradione[18]	35.5 (2.6)	1.2956	–0.92	0.00	150.19	12.03
21	azadiradione pp	34.7 (4.3)	1.5297	–0.82	0.00	132.35	12.28
22	epoxy azadiradione[18]	50.4 (3.1)	1.3068	–0.86	0.08	160.61	10.77
23	epoxy azadiradione pp	29.4 (5.8)	1.3280	–0.75	0.08	136.95	11.12
24	azadirone	48.3(3.2)	1.2052	–1.08	0.00	155.18	11.38
25	homoazadiradione	60.0 (6.0)	1.2956	–1.13	0.15	155.10	11.28
26	salannin[4]	36.3 (2.7)	1.3821	–0.77	0.15	96.28	8.91
27	salannin pp1	29.1(0.6)	1.3572	–0.68	0.23	105.00	9.50
28	salannolide	26.3 (1.5)	1.3398	–0.68	0.23	105.83	9.50
29	salannin pp	25.1 (1.3)	1.3572	–0.68	0.23	110.46	9.40
30	salannin MW	46.3 (2.1)	1.3950	–0.68	0.23	15.32	9.56
31	2′3′ dehydro salannol	52.1 (6.5)	1.4367	–0.79	0.23	34.10	9.60
32	3-oxosalannin	42.9 (3.5)	1.3428	–0.76	0.23	96.06	8.95
33	desacetyl salannin	44.67(4.7)	1.4365	–0.79	0.15	35.20	9.59
34	desacetyl salannolide	28.44(1.4)	1.4056	–0.70	0.23	12.33	9.21
35	desacetyl salannin MW	40.35(6.8)	1.4472	–0.70	0.23	18.17	9.52
36	nimbin[4]	33.7 (2.4)	1.3356	–0.54	0.15	81.99	8.83
37	nimbinolide	26.7(3.2)	1.4001	–0.44	0.15	105.83	9.00
38	nimbin pp	19.9 (2.3)	1.3921	–0.44	0.15	111.33	9.00
39	nimbin MW	42.9 (3.5)	1.3645	–0.44	0.15	103.19	8.92
40	desacetylnimbin[4]	34.7(0.7)	1.3764	–0.50	0.15	150.32	8.74
41	desacetylnimbin MW	35.1 (3.2)	1.3769	–0.54	0.15	101.76	9.03
42	nimbolide	37.0 (1.8)	1.3154	–0.54	0.23	30.90	9.68
43	6-oxonimbin	42.3(2.2)	1.3047	–0.47	0.15	82.76	8.43
44	azadirachtin-A[18]	27.5 (4.0)	1.0840	–0.15	0.38	62.84	9.49
45	azadirachtin-B[18]	26.7 (1.7)	1.0763	–0.31	0.38	81.04	8.50
46	azadirachtin-D[18]	28.9 (3.2)	1.1104	–0.24	0.38	77.73	8.52
47	azadirachtin-H[18]	30.5 (3.0)	1.1256	–0.25	0.38	66.32	8.88
48	azadirachtin-I[18]	32.2 (6.4)	1.1383	–0.39	0.38	79.25	8.48
49	3-tigloyl meliacarpin	13.3 (1.6)	1.1202	–0.48	0.38	81.13	8.49
50	azadirachtol	6.1 (2.4)	1.2461	–0.54	0.38	81.33	8.48
51	3α-acetoxy-1α-hydroxyazadirachtol	4.2 (0.7)	1.1784	–0.64	0.38	81.02	8.50
52	gedunin	37.3 (4.4)	1.3132	–0.77	0.15	141.63	12.02
53	swietenine	49.1 (4.9)	1.3626	–0.82	0.68	137.89	8.71
54	swietenine pp	40.2 (2.6)	1.3729	–0.73	0.68	150.27	8.82
55	methylangolensate	65.3(2.7)	1.3782	–0.69	0.31		
56	methylangolensate MW	35.2(3.3)	1.3854	–0.62	0.31		

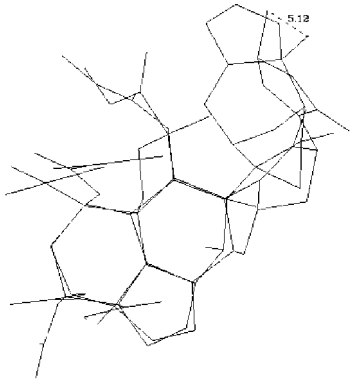
[a] Abbreviations used: PFI, percentage feeding index; K'w, hydrophobicity constant; *O*, oxidation state; *S*, skeletal specialization; OF, orientation of furan ring; dist. C-3/C-23, distance between C-3 and C-23.

that of the parent molecule. With the C-seco limonoids the reverse was found true. As modification of the furan moiety was shown critical for antifeedant activity, attempts were made to find whether there is any correlation of furan orientation (A_1°) in relation to the rest of the molecule to the antifeedant effect. In intact limonoids and their photoproducts, the dihedral angle was found to be in the range of 157−129°, whereas in the case of C-seco limonoids and their photoproducts the dihedral angles are in the range of 96−105° (**Table 1**). It is possible that there may be an optimal orientation of modified furan for maximizing antifeedant activity pointing toward 129−105°. This needs further confirmation through correlation of a large number of limonoids with modified furans (having different dihedral angles) to antifeedant activity.

In the intact apoeuphol limonoids antifeedant activity was correlated to a 14,15-epoxide and either a 19/29 lactol bridge or a cyclohexenone A-ring (*3*). The intact and C-seco limonoids and their photoproducts presently studied possess a carbonyl function at C-3 (except nimbin and azadirachtin-A). It was observed that hydroxylation of C-23 (furan) due to photomodification of intact and seco limonoids results in a change of distance between C-3 and C-23. As these positions appear critical for antifeedant activity, it was decided to quantitate the distance between C-3 and C-23. Because the stereochemistry of −OH in the furan moiety was not determined in the present investigation (whether above or below), the distance was calculated for both the epimers ($Å_1$) and linear regression analyses with antifeedant activity are presented (**Table 1**). Azadirachtin-A and its congeners are highly modified C-seco compounds in which the furan is modified to a dihydrofuranyl moiety in which the free hydroxyl occurs in C-20 in β configuration. Hence, for correlation of antifeedant activity, C-3

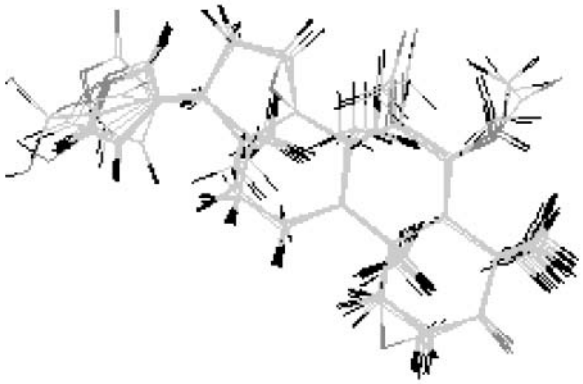
to C-20 distances are presented for congeners of azadirachtin-A. There is an increase in distance between C-3 and C-23 in all the photoproducts compared to that of the respective parent molecule, and this increase correlated well with increased antifeedant activity of the respective photoproduct. Comparing the antifeedant activity (%) of all the limonoids against the measures of C-3−C-23 distance, positive correlation was not noted. It is hence possible to conclude that this distance cannot be a general measure of antifeedant activity of a given limonoid molecule. Instead, it would be appropriate to surmise that introduction of a hydroxyl function at C-23 will bring about increased hydrophilicity due to furan modification. In the azadirachtin and its congeners, hydroxyl function is found in the dihydrofuranyl moiety, which falls at the same distance as that of the hydroxyl in the furan of photomodified limonoids. An overlap of photomodified salannin and azadirachtin A clearly illustrates the close similarity in C-3−C-23 distance and the orientation (Overlap diagram I). In overlap, the −OH group in salannolide deviated by 5.12 Å compared to that of azadirachtin-A. This distance may not be critical, as both azadirachtin-A and salannin have C-8−C-14 and C-17−C-20 bonds that are free rotating, respectively, in these two molecules. This may in part explain the almost identical antifeedant activities of salannolide and azadirachtin-A (**Table 1**).

Overlap diagram I

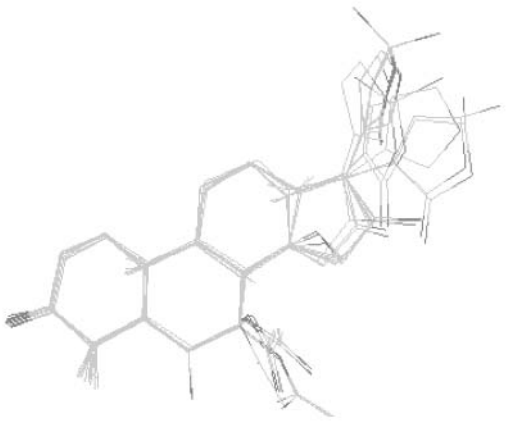


Using molecular modeling, relaxed bond distance between oxygen atoms at C-3 and C-20 of the azadirachtin molecule was found comparable to that of 20-*β*-hydroxyecdysone, which is the moulting hormone in insects. This feature was assumed to have significance in binding to ecdysone receptors in the insects (*28*). The present investigation did not target moulting for bioactivity studies. The α−α enone function of the A-ring and the epoxidation of the C-20−C-21 (**16**) double bond in the furan moiety have been shown to be critical for antifeedant activity also (**Table 1**). Considering epoxidation results in a change of the distance between these two active sites, it was presumed that this may have an impact on antifeedant activity as well. The results show only marginal change of distance in C-3−C-23 bond distances ($Å_2$) and such marginal changes did not correlate with the antifeedant activity of the substrate limonoid and its photoproduct (**14** and **16**). (Overlap diagrams 2 and 3).

Terpenoids in general have been characterized as having lipophilic properties, but are water soluble at biologically active concentrations, the ecological implications of which have been discussed in detail by Weidenharner et al (*30*). It is appropriate to study the changes in hydrophobicity of the substrate limonoids and the photoproducts as a measure of changes in antifeedant activity. The hydrophobic constant (log K'_w) values of photo-



Overlap diagram 2 (1-10)



Overlap diagram 3 (12-16 and 19-23)

products were more compared to their respective substrates (**Table 1**) which in turn reflects increased hydrophilicity of the photoproducts. Concomitantly, the antifeedant activity (%) of the photoproducts also increased indicating positive correlation. This is in conformity with the observation that insect antifeedant activity of terpenoids has been linked to the oxygenation, which may maintain sufficient polarity to allow aqueous diffusion to the taste receptor protein in the chemosensory sensilla (*22*). The only exception to this trend was salannin and its photoproducts, wherein a reduction in hydrophobic constant was noticed. Studies with a large number of limonoids may throw more light on the optimal hydorphobicity for maximizing antifeedant activity.

Based on molecular modeling, common binding features for high antifeedant activity among polycyclic terpenoids were identified (*21*) which included an epoxide, π bonding sites separated by 5−6 Å, one or more electronegative oxygen centers, and polyoxygenation to maintain sufficient polarity. The present study clearly illustrates that increased oxidation states may not result in increased activity and that oxygenation has to be at specific loci in the molecule such as −OH introduction in the intact furan moiety and possibly in the A ring. Interestingly, it is found that C-23−OH and possibly C-3−OH must be free (to bind to the receptor site). The oxidation of the furan ring did increase the electronegativity of the oxygen center, possibly facilitating proper binding to the receptor site. Compounds **10** and **16** have an epoxide in the furan (at C-22−23 and at C-20−21 respectively) due to the photomodification. Although compound **10** showed only a marginal increase in antifeedant activity compared to that of its parent compound **1**, the nimonol photoproduct (**16**) showed substantial increase in

antifeedant activity, indicating the importance of epoxide position within the furan ring.

The most active limonoid antifeedant could be either an intact apoeuphol compound or a C-seco compound with a hydroxylated furan (a −OH that may overlap with a C-20−OH in azadirachtins) and a dihydroxy A-ring. Efforts are underway to modify a limonoid and confirm the aforesaid conclusion.

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