Insect Antifeedant Ryanodane Diterpenes from *Persea indica*

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The new ryanodane diterpenes cinnzeylanone, ryanodol 14-monoacetate, and *epi*-cinnzeylanol have been isolated from *Persea indica* (Lauraceae). The structure of cinnzeylanone has been determined by X-ray analysis. These compounds proved to be antifeedants against *Spodoptera litura*. Cinnzeylanol and ryanodol were the most active antifeedants of this plant, with a range of activity close to that of ryanodine. This is the first report on the antifeedant effects of this class of diterpenes. Their structure−activity relationships and possible mode of action are discussed.

Keywords: *Ryanodane diterpenes; cinnzeylanone; ryanodol 14-monoacetate; epi-cinnzeylanol; antifeedants*

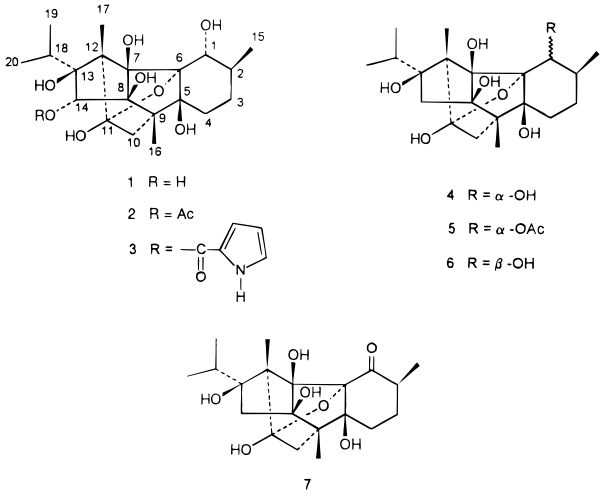
INTRODUCTION

Botanical pesticides have the advantage of providing novel modes of action that can reduce the risk of cross-resistance (Berenbaum, 1989) as well as offering new leads for the design of target-specific molecules.

As a part of our ongoing search for natural agrochemicals from endemic Canarian plants, we have thus far investigated the potential of species in the Lauraceae family. In a previous work we have reported the isolation of the toxic diterpenes ryanodol (**1**) and cinnzeylanol (**4**) from *Persea indica* (González-Coloma et al., 1990, 1993), which is one of the dominant species of the Canarian laurel forest, a relict of the Tertiary flora (Bramwell, 1976). We have also studied the ecological relation of these diterpenes with wild rats and its insecticidal effects on *Macaronesia fortunata* and *Heliothis armigera* (González-Coloma et al., 1990, 1992). Furthermore, a recent screening has shown that an extract of this plant is also a potent antifeedant against the polyphagous crop pest *Spodoptera litura* (González-Coloma et al., 1994). Now, following the study of this plant we have isolated the known diterpene cinnzeylanine (**5**) (Isogai et al., 1976, 1977) and the three novel diterpenes cinnzeylanone (**7**), ryanodol 14-monoacetate (**2**), and *epi*-cinnzeylanol (**6**) and shown the antifeedant activity of these ryanoids against *S. litura*. Their structure−activity relationships and possible mode of action are also discussed. Ryanodine (**3**), an insecticidal alkaloid with a ryanodane diterpene skeleton (Crosby, 1971), was included in this work for structure−activity comparison.

MATERIALS AND METHODS

Apparatus, Chromatography, and Chemicals. Melting points were taken on a Kofler hot-plate apparatus (Reichert-Jung) and are uncorrected. Rotatory circular chromatographies were made in a Chromatotron from Harrison Research. ^{1}H and ^{13}C NMR spectra were recorded on Bruker WP200SY and AMX400 instruments. Mass spectra were taken at 70 eV (probe) in a Hewlett-Packard 5995 and a Shimadzu Q2000 and high resolution mass spectra in a VG Micromass ZAB-2F. Infrared spectra were recorded in a Perkin-Elmer 1605.



The substances were crystallized from petroleum ether−EtOAc except where otherwise indicated. Dry column chromatographies were made in silica gel Merck 0.02−0.063 mm. Ryanodine was purchased from Sigma.

Plant Material. Extraction and Isolation of Compounds. *P. indica* branches were collected at Monte de Las Mercedes, Tenerife, in March. Air-dried, chopped, aerial parts (1.3 kg) were extracted with ethanol in a Soxhlet apparatus. The cold extract was filtered and then concentrated *in vacuo* to afford a syrupy gum (307 g). This syrup was treated with EtOAc and the solution separated by filtration. The solvent was evaporated off and the residue (138 g) chromatographed in silica gel (500 g) and eluted with petroleum ether−EtOAc mixtures. In this way, after several rechromatographies in "dry column" mode and using also rotatory circular chromatography, the following diterpenes were obtained in polarity order: cinnzeylanone (**7**) (290 mg), cinnzeylanine (**5**) (110 mg), *epi*-cinnzeylanol (**6**) (16 mg), cinnzeylanol (**4**) (280 mg), ryanodol 14-monoacetate (**2**) (53 mg), and ryanodol (**1**) (245 mg).

Spectral Data and Reactions. *Cinnzeylanol* (**4**): mp 140−141°C (from MeOH); $[M]^+$ at m/z 384.2157 ($C_{20}H_{32}O_7$ requires 384.2166); ^{1}H NMR (200 MHz, CD_3OD) δ 0.86 and 1.31 (3H, s, H-16 and H-17), 0.95 (3H, d, J = 6 Hz, H-15), 0.92 and 0.97 (each 3H, d, J = 6 Hz, H-19 and H-20), 1.76 and 2.40

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(each 1H, d, J = 15 Hz, H-14), 3.80 (1H, d, J = 10 Hz, H-1); EIMS m/z (rel int) 384 $[M]^+$ (2), 3.66 (5), 348 (17), 341 (14), 330 (69), 323 (100), 315 (17), 305 (24), 289 (75), 287 (20), 271 (22), 259 (20), 223 (13), 217 (11), 195 (15).

Cinnzeylanine (**5**): ^{1}H NMR (400 MHz, CD_3OD) δ 0.80 (3H, d, J = 6 Hz, H-15), 0.84 and 1.29 (each 3H, s, H-16 and H-17), 0.91 and 0.96 (each 3H, d, J = 6.4 Hz, H-19 and H-20), 1.54 (2H, m, H-3), 1.69 and 2.35 (each 1H, d, J = 16 Hz, H-14), 1.71 and 1.82 (each 1H, d, J = 14 Hz, H-10), 1.85 (1H, m, H-18), 1.98 (1H, m, H-2), 2.01 (3H, s, −OAc), 2.09 (1H, m, H-4), 5.21 (1H, d, J = 11 Hz, H-1); EIMS m/z (rel int) 366 $[M - AcOH]^+$ (80), 348 (43), 320 (21), 305 (33), 287 (28), 269 (25), 262 (51), 259 (32), 245 (15), 235 (50), 234 (44), 233 (88), 216 (24).

Cinnzeylanone (**7**): IR (film) ν_{max} 3350, 1700, 1400, 1320, 1100, 1040 cm^{-1}; $[M]^+$ at m/z 382.2000 ($C_{20}H_{30}O_7$ requires 382.1991); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 and 1.02 (each 3H, d, J = 6 Hz, H-19 and H-20), 0.99 (3H, d, J = 8 Hz, H-15), 1.00 and 1.24 (each 3H, s, H-16 and H-17), 1.56 (1H, ddd, J = 1.7, 4, and 12 Hz, H-4), 1.80 (1H, m, H-18), 1.82 and 1.99 (each 1H, d, J = 14 Hz, H-10), 1.86 and 2.53 (each 1H, d, J = 16 Hz, H-14), 2.40 (1H, td, J = 5 and 12 Hz, H-4), 2.96 (1H, m, H-2), 5.71 (1H, br s, OH), 6.65 (1H, br s, OH); ^{1}H NMR (400 MHz, CD_3OD) δ 0.86, and 1.13 (each 3H, s, H-16 and H-17), 0.90 and 0.91 (each 3H, d, J = 5 Hz, H-19 and H-20), 0.92 (3H, d, J = 8 Hz, H-15), 1.70 and 2.40 (each 1H, d, J = 14 Hz, H-10), 1.71 and 1.92 (each 1H, 16 Hz, H-14), 1.43 (1H, ddd, J = 1.7, 4 and 12 Hz, H-4), 1.83 (1H, m, H-18), 2.41 (1H, m, H-4), 2.91 (1H, m, H-2); EIMS m/z (rel int) 382 $[M]^+$ (26), 364 (12), 346 (14), 321 (100), 303 (15), 301 (9), 293 (16), 279 (10), 275 (26), 257 (18), 235 (31), 233 (15), 223 (25), 215 (15), 209 (13), 207 (14), 205 (20), 194 (30), 191 (18), 154 (56).

epi-Cinnzeylanol (**6**): $[M - 2H_2O]^+$ at m/z 348.1929 ($C_{20}H_{28}O_5$ requires 348.1936); ^{1}H NMR (400 MHz, CD_3OD) δ 0.88 and 1.25 (each 3H, s, H-16 and H-17), 0.92 (3H, d, J = 6 Hz, H-15), 0.97 (6H, d, J = 6 Hz, H-19 and H-20), 1.39 (1H, m, H-3), 1.42 (1H, m, H-4), 1.55 (1H, ddd, J = 4, 13, and 13 Hz, H-3), 1.74 and 1.82 (each 1H, d, J = 14 Hz, H-10), 1.75 and 2.39 (each 1H, d, J = 16 Hz, H-14), 1.90 (1H, m, H-18), 2.01 (1H, m, H-2), 2.12 (1H, td, J = 5 and 12 Hz, H-4), 3.82 (1H, d, J = 2.4 Hz, H-1); EIMS m/z (rel int) 348 $[M - 2H_2O]^+$ (4), 330 (9), 323 (9), 315 (2), 195 (13), 149 (23), 94 (100).

Ryanodol (**1**): mp 345−347 °C (from MeOH−H_2O); $[M]^+$ at m/z 400.2090 ($C_{20}H_{32}O_8$ requires 400.2097); ^{1}H NMR (400 MHz, CD_3OD) δ 0.89 and 1.33 (each 3H, s, H-16 and H-17), 0.95 and 1.00 (each 3H, d, J = 6 Hz, H-19 and H-20), 1.05 (3H, d, J = 6 Hz, H-15), 1.66 and 1.87 (each 1H, d, J = 16 Hz, H-10), 3.74 (1H, s, H-14), 3.80 (1H, d, J = 10 Hz, H-1); EIMS m/z (rel int) 400 $[M]^+$ (7), 382 (2), 357 (16), 339 (100), 321 (63), 303 (49), 293 (36), 261 (25), 257 (14), 247 (18), 243 (20), 229 (11), 195 (10), 165 (9), 155 (12).

Ryanodol 14-Monoacetate (**2**): $[M - C_3H_7 - H_2O]^+$ at m/z 381.1539 ($C_{19}H_{25}O_8$ requires 381.1549); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.82 (3H, d, J = 7 Hz, H-15), 1.03 and 1.05 (each 3H, d, J = 6 Hz, H-19 and H-20), 1.09 and 1.39 (each 3H, s, H-16 and H-17), 1.83 and 1.98 (each 1H, d, J = 14 Hz, H-10), 2.10 (3H, s, −OAc), 3.89 (1H, d, J = 10 Hz, H-1), 5.09 (1H, s, H-14); ^{1}H NMR (400 MHz, CD_3OD) δ 0.78 (3H, d, J = 7 Hz, H-15), 0.90 and 1.32 (each 3H, s, H-16 and H-17), 0.95 and 0.98 (each 3H, d, J = 6 Hz, H-19 and H-20), 1.70 and 1.88 (each 1H, d, J = 14 Hz, H-10), 2.01 (3H, s, −OAc), 3.74 (1H, d, J = 11 Hz, H-1), 5.04 (1H, s, H-14); EIMS m/z (rel int) 381 $[M - C_3H_7 - H_2O]^+$ (2), 363 (5), 346 (3), 328 (3), 318 (2), 303 (5), 195 (100), 177 (17), 163 (10), 154 (21), 152 (25).

Reduction of Cinnzeylanone (**7**). Compound **7** (22 mg) in MeOH (4 mL) was treated with $NaBH_4$ (10 mg) at room temperature for 3 h. The solution was poured into H_2O and acidified. The product was recovered in EtOAc and chromatographed in silica gel. Elution with EtOAc gave a possible borate ester (9 mg): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.94 and 1.24 (each 3H, s), 0.95 (3H, d, J = 6 Hz, H-15), 0.97 and 0.98 (each 3H, d, J = 6 Hz, H-19 and H-20), 1.82 and 2.40 (each 1H, d, J = 16 Hz, H-14), 4.00 (1H, d, J = 3 Hz, H-1). Further elution afforded **6** (1.6 mg), which was identical with the natural *epi*-cinnzeylanol.

Crystal Data of Cinnzeylanone (**7**): $C_{20}H_3O_7$, M_r = 382.453; monoclinic, a = 13.306(1) Å, b = 13.577(1) Å, c = 12.095(1) Å, β = 112.434(2)°, V = 2019.7(3) Å^3, space group $P2_1$; Dc = 1.2578 mg(10^{-3}), μ = 7.435 cm^{-1}, F(000) = 824.0.

A colorless well-formed crystal of approximate dimensions 0.40 × 0.30 × 0.35 mm was used for data collection. The lattice parameters were obtained from least-squares analysis of 45 reflections with 10 < Θ < 45°, from graphite monochromated Cu Kα radiation on a Philips PW 1100 diffractometer. Intensity data of 3590 unique reflections were collected by $\omega-2\Theta$ scan technique, scan speed 0.050(°) seg, with Θ between 2° and 65°; 3393 unique reflections with $I > 2\sigma(I)$ were considered as observed. Intensity and orientation standards were measured again every 90 reflections, and no significant decomposition or movement of the crystal was observed. Corrections were made for Lorentz and polarization but not for absorption effects.

The structure was solved by direct methods (SIR88) (Burla et al., 1989) and Fourier synthesis, and refinement was by least squares using anisotropic thermal parameters for the heavier atoms. Hydrogen atoms were located from difference maps and refined isotropically. The weighting scheme used in the last cycles of refinement was chosen not to give dependence on $\langle w\Delta^2F\rangle$ over ranges of $\langle F_o\rangle$ and $\langle \sin\Theta/\lambda\rangle$. Maximum peak height in the final difference Fourier map was 0.191 Å^{-3}. Final R and R_w values were 4.4 and 5.3, respectively. All calculations were performed on a Vax 6410 computer and with X-ray 76 programs (Stewart et al., 1976). The program used for plotting molecular and crystal structures was PLUTO (Motherwell and Clegg, 1978).

Lists of atomic coordinates, thermal parameters, structure factors, bond lengths, bond angles, and torsion angles have been deposited at the Cambridge Crystallographic Data Centre (U.K.).

Insect Bioassays. *S. litura* (Noctuidae) larvae were reared on artificial diet (Insecta LF, Nihon Niosan Kogyo) at 27 °C, 70% relative humidity and 16:8 h photoperiod. Two different bioassays were performed.

Leaf-Disk Bioassay. This bioassay was designed to quantify the feeding deterrency of the plant extract and the pure compounds. The bioassay was performed with third-instar larvae as described by Escoubas et al. (1993). A feeding index (FI) was calculated for each treatment at 1000 ppm for comparison of the activities, and an arbitrary level (FI < 25) was used as the criterion to determine very effective deterrents. FI = 100 × %T(%T + %C); %T is the percentage of treated disks consumed, and %C is the percentage of control disks consumed. The index varies from 0 (total inhibition) to 100 (total stimulation).

A dose range of 10, 25, 50, 75, 100, 250, 500, 750, and 1000 ppm was used with the effective antifeedants to estimate their percent feeding inhibition [1− (%T/%C) × 100] and calculate their relative potencies (EC_{50}, effective dose to give a 50% feeding inhibition) with probit analysis (Finney, 1976).

Bioautography TLC Bioassays. This bioassay-guided procedures provided a fast method for the location and identification of plant antifeedants and was performed as described by Escoubas et al. (1992).

Toxicity Bioassays. A toxicity bioassay was performed as described by González-Coloma et al. (1990) by intraperitoneal injection of five laboratory mice with 5 mg of cinnzeylanone (**7**) (25 mg/kg), to test the toxicity of this insect antifeedant. The toxicity of cinnzeylanol (**4**), ryanodol (**1**), and ryanodine (**3**) had been previously determined (Waterhouse et al., 1987; González-Coloma et al., 1990).

RESULTS AND DISCUSSION

Determination of the New Structures. The high-resolution mass spectrum of the new diterpene cinnzeylanone (**7**) showed the molecular ion at m/z 382.2000, indicating a molecular formulaof $C_{20}H_{30}O_7$. Its IR spectrum exhibited absorptions of hydroxyl and carbonyl groups. The ^{1}H NMR spectra were run in $CDCl_3$ and CD_3OD, showing that several singlets observed in the

Table 1. Hydrogen Bonds (7)

X--H···Y			X·--H	X···Y	H···Y	X--H··Y
O7a	H7a	O1a(1)	0.922	2.713	1.845	156(°)
O5a	H7a	O8a(1)	0.930	2.573	1.724	150
O8a	H8a	O13b(3)	0.815	2.765	1.946	168
O11a	H11a	O5b(1)	0.938	2.801	1.879	167
O13a	H13a	O7b(3)	0.913	2.970	2.247	136
O7b	H7b	O1b(1)	0.968	2.736	1.873	147
O5e	H5b	O8b(1)	0.989	2.603	1.642	163
O8b	H8b	O13a(4)	1.020	2.931	1.923	169
O11b	H11b	O5a(1)	0.752	2.753	2.012	169
O13b	H13b	O7a(4)	0.991	2.816	2.268	114

Symmetry Code: (1) X,Y,Z; (3) $1 - X$, $1/2 + Y$, $1 - Z$; (4) $1 - X, Y - 1/2, 1 - Z$

first spectrum were due to hydroxyl groups. In both spectra two angular methyls, an isopropyl group, a secondary methyl, and two isolated methylene groups were observed. The ^{13}C NMR spectrum showed the presence in the molecule of five methyls, four methylenes, two methines, eight tetrasubstituted carbons, and one carbonyl group. The carbon resonances have been assigned using 2D NMR spectra, HMQC and HMBC, and are described in Table 1. Thus, we gave this compound a structure of ryanodane with a pentacyclic skeleton. The presence in the molecule of two AB systems at 1.82 and 1.99 ($J = 14$ Hz), the former, and 1.86 and 2.53 ($J = 16$ Hz), the latter, characteristic of two isolated methylene groups assigned to H-10 and H-14, respectively, indicated that the carbonyl group was in the cyclohexane ring. The resonance of the C-2 at δ 41.6 compared to 35.3 in cinnzeylanol (**4**) pointed to C-1 for the oxo group. When all of these data were taken into consideration, the structure **7** was assigned to this compound and named cinnzeylanone.

To confirm this structure and to determine the stereochemistry of the methyl at C-2, this substance was subjected to X-ray analysis. The structure **7** was thus confirmed.

The results of this study showed two independent molecules in the asymmetric unit, which have been assigned as A and B. Both molecules have similar and identical conformations. The crystal structure is shown in Figure 1. The molecule resembles a cage consisting of two six-membered rings and three five-membered rings. The six-membered rings have some distorted chair conformations, showing distortions from the ideal symmetry with endocyclic dihedral angles between 45° and 87°. The five-membered rings have an envelope and half-chair conformation. The stereochemistry of the molecule shows that the hydroxyl groups O-5, O-7, O-8, and O-13 are β-configured with respect to the rings to which they are linked, while hydroxyl O-11 is α-configured. The several asymmetric carbon atoms have the following configurations: C-2(*R*), C-5(*R*), C-6(*S*), C-7(*S*), C-8(*S*), C-4(*R*), C-12(*R*), and C-13(*R*).

The packing of the crystal is totally determined by a network of hydrogen bonds as shown in Figure 2 and summarized in Table 1.

The molecules of **7** are arranged in the crystal as strips of A and B molecules along the *b* axis strongly linked by H-bonds giving rise to a secondary structure. The strips are fixed along the a axis (through the O-5 and O-11 atoms in both molecules A and B), forming the tertiary structure; those packed along the *c* axis by van der Waals forces form the crystal (fourth structure).

Another new compound obtained from this plant was the diterpene **6**, which showed a ^{13}C NMR spectrum (Table 2) similar to that of cinnzeylanol (**4**), with the

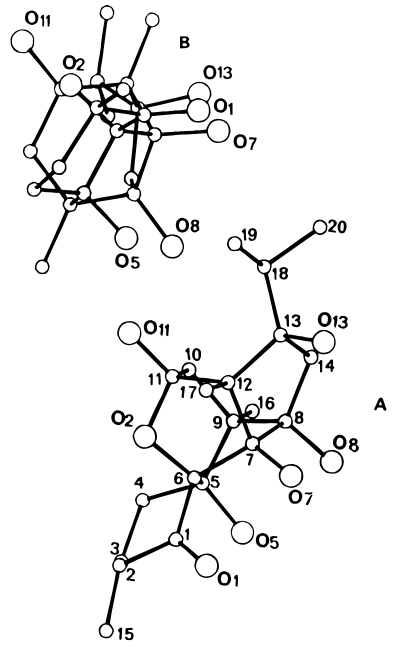

Figure 1. PLUTO drawing of molecules A and B with atomic numbering.

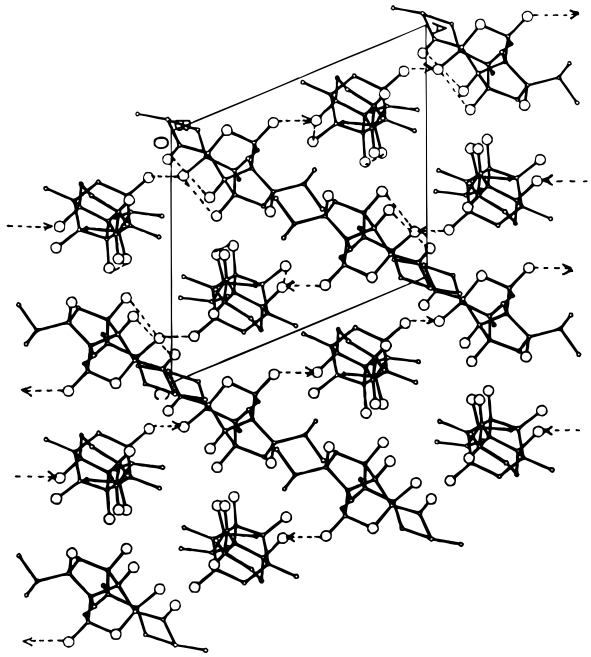

Figure 2. PLUTO plot of the crystal packing along *b*. The dashed lines indicate hydrogen bonds.

major differences in the chemical shifts of the cyclohexane rings. In the mass spectrum the molecular ion was not observed and that of the higher mass appeared at *m/z* 348, which was originated by loss of two molecules of water. Its ^1H NMR spectrum showed the geminal proton to a hydroxyl group as a doublet at δ 3.82 ($J = 2.4$ Hz). Thus, we think that this compound must be the epimer of cinnzeylanol at C-1 (**6**). This fact was confirmed by sodium borohydride reduction of cinnzeylanone (**7**), which gave a product (**6**) identical with the natural *epi*-cinnzeylanol. A less polar compound, also obtained in this reaction, was a possible borate ester (Jefferies and Casida, 1994).

A diterpene monoacetate was also isolated from this plant and characterized as the new natural derivative ryanodol monoacetate (**2**). Its ^1H NMR spectrum showed the geminal proton to the acetoxyl group in C-14 as a

Table 2. ^{13}C NMR Data of 1, 4, and 5 (CDl_3OD) and 2, 6, and 7($CDCl_3$)

C	1	2	4	5	6	7
1	72.9	72.0	72.6	74.4	74.8	214.9
2	35.2	34.5	35.3	34.2	33.0	41.6
3	29.6	28.9	29.5	29.2	23.3	31.0
4	27.2	26.6	27.3	27.1	26.9	26.5
5	85.9	84.3	86.2	86.3* [a]	84.5*	81.3
6	87.9*	87.1*	87.2	86.7*	82.2*	88.2
7	97.9	96.4	98.3	97.6	96.6	97.6
8	87.6*	86.7*	90.2	90.2	88.8	88.2
9	n.o.	48.0	48.9	n.o.	48.1	47.6
10	42.4	41.2	43.4	43.3	42.0	41.7
11	102.2	100.9	102.4	102.4	101.7	101.7
12	64.4	63.1	66.2	66.3	67.2	64.7
13	82.9	82.5	83.5	83.5	80.5*	81.9
14	75.1	75.2	49.8	49.6	48.9	50.5
15	18.5	17.7	18.8	18.4	17.0	13.0
16	11.1	10.5	11.1	11.1	10.1	10.4
17	9.5	8.5	9.7	9.7	9.7	9.5
18	34.0	32.9	34.2	34.1	31.1	32.9
19	18.2	18.0	19.0	18.8	18.3	18.2
20	19.0	18.2	19.1	19.1	18.4	18.3

[a] Values marked with asterisks may be interchanged.

Table 3. Yield and Feeding Indices of the Ethanolic Extract and the Pure Ryanoids from *P. indica* against *S. litura* Larvae in a Choice Leaf-Disk Bioassay

test substance (1000 ppm)	% yield (g of dry wt)	index av (SE)
extract	23.0	0.00 (0.00)
ryanodol (**1**)	40.74×10^{-3}	0.51 (0.51)
cinnzeylanol (**4**)	47.56×10^{-3}	7.28 (6.51)
cinnzeylanone (**7**)	31.93×10^{-3}	9.41 (1.72)
cinnzeylanine (**5**)	19.62×10^{-3}	23.41 (8.05)
epi-cinnzeylanol (**6**)	9.47×10^{-3}	6.60 (4.26)
ryanodol 14-monoacetate (**2**)	0.5×10^{-3}	55.64 (5.72)

singlet at δ 5.09, while in ryanodol the geminal hydrogen to the corresponding alcohol appeared at δ 3.74. The ^{13}C NMR spectrum (Table 2) was similar to that of ryanodol (**1**). This monoacetate (**2**) was hydrolyzed by the acid medium to ryanodol (**1**) when it was left in the NMR tube for several weeks (solvent $CDCl_3$).

Biological Activities. Positive results were obtained from the TLC bioautography test performed with a crude extract of *P. indica*, and six antifeedant ryanodane diterpenes were isolated. Table 3 shows that the most abundant chemical was cinnzeylanol (**4**) followed by ryanodol (**1**), cinnzeylanone (**7**), cinnzeylanine (**5**), and *epi*-cinnzeylanol (**6**), while ryanodol 3-monoacetate (**2**) was the least abundant of these compounds. All of the ryanoids gave an FI < 23 (significant feeding inhibition >50%) with the exception of compounds **2** and **5**.

The dose−response data (Figure 3; Table 4) indicate that ryanodine (**3**) had the highest activity, followed by diterpenes **6**, **4**, **1**, and **7**. Compounds **2** and **5** showed the lowest effective doses among these ryanoids. Azadirachtin, one of the strongest known natural antifeedants, was included in the table as a positive control. The mammalian toxicity of the major components ranked similarly to the antifeedant activity (Table 5).

The structure−activity relationships of the ryanodane diterpenes studied here showed that both C-14 and C-1 substituents play an important role in their antifeedant activity. Acetylation of this center as in **2** or **5** results in loss of activity, while the pyrrolecarboxylate at C-14 (**3**) confers higher potency. Furthermore, it has been shown that the mortality rate caused by cinnzeylanol (**4**) was twice that caused by cinnzeylanine (**5**), when tested against *Bombyx mori* (Isogai et al., 1977). Additionally, studies carried out on the structure−activity relationships of ryanodine (**3**) and several derivatives and degradation products pointed to the importance of the C-14 substituent and the stereochemistry and hydrophobicity of the cyclohexane ring and its substituents, including the hydroxyl group at C-1, in the inhibition of the calcium release channel and knockdown effects on houseflies (Waterhouse et al., 1987; Jefferies and Casida, 1994).

The biological activities of the known ryanoids have been studied to some extent. Cinnzeylanol (**4**) and cinnzeylanine (**5**) were shown to have anticomplement activity (Yagi et al., 1980) and were also toxic to several insect species (Isogai et al., 1977). Ryanodine (**3**) is a very potent nonselective probe for the Ca^{2+} release channels of housefly, cockroach, and mouse muscle membranes and also a nonselective injected toxicant for insects and mammals (Waterhouse et al., 1987; Jefferies et al., 1992).

Ryanodol (**1**) is very toxic to houseflies and cockroaches [25−50% as effective as ryanodine (**3**)] but not to mice (<0.5% as toxic as ryanodine) (Waterhouse et

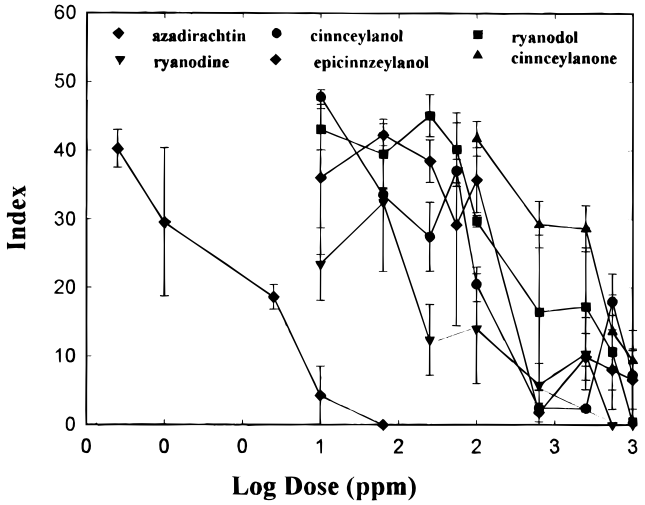

Figure 3. Dose−response curves of four ryanoids from *P. indica* against third-instar *S. litura* larvae in a choice leaf-disk bioassay. Data are represented as mean ± SE (2−5 experiments for each treatment, $n = 20$ for each experiment).

Table 4. Structure−Activity Relationships of the Test Compounds Based On Their Relative Potencies

compd	substituents C-14(α)	substituents C-1	log EC_{50}[a] (ppm)	95% limits (lower, upper)
6	H	α-OH	1.891	1.51, 2.27
4	H	β-OH	1.934	1.44, 2.42
1	OH	β-OH	2.120	1.79, 2.44
7	H	=O	2.598	2.35, 2.84
5	H	β-OAc	>3.0	
2	OAc	β-OH	>3.0	
3	PC[b]	β-OH	1.240	0.78, 1.70
AZA[c]			0.247	−0.08, 0.57

[a] Effective dose to give a 50% feeding deterrency (FI = 25). [b] PC, pyrrole-2-carboxylate. [c] AZA, azadirachtin, has been included as a positive control (Lajide et al., 1993).

Table 5. Mammalian Toxicity (Mouse) of the Major Diterpene Antifeedants

compd	LD_{50}[a] (mg/kg)	compd	LD_{50}[a] (mg/kg)
cinnzeylanol (**4**)	11.988[b]	cinnzeylanone (**7**)	>25.0
ryanodol (**1**)	17.918[c]	ryanodine (**3**)	0.1[b]

[a] Effective dose to give a 50% mortality. [b] From González-Coloma et al. (1990). [c] From Waterhouse et al. (1987).

al., 1987; González-Coloma et al., 1990; Jefferies et al., 1992). It is also less than 1% as active as ryanodine at the [^{3}H]ryanodine binding site in both insects and mice (Lehmberg and Casida, 1994). On the basis of the similarity of the ryanodine binding site between mammals and insects, these authors suggest that ryanodol may have a different binding site with a greater effect on insects.

Our data showed that the antifeedant potency ratios between ryanodine (**3**) and the ryanodane diterpenes varied between 1.52 and 2.10 versus a range of potency ratios for mice toxicity of between 180 and 250. These data suggest a selective activity of the ryanoid diterpenes for insects in agreement with the above-mentioned hypothesis. Additionally, this is the first time that an antifeedant action is reported for this class of natural products.

In summary, six diterpene ryanoid antifeedants have been isolated from *P. indica*. *epi*-Cinnzeylanol (**6**), cinnzeylanol (**4**), and ryanodol (**1**) had the highest antifeedant potency followed by cinnzeylanone (**7**), while cinnzeylanine (**5**) and ryanodol 14-monoacetate (**2**) had the weakest effects. The comparison of the mammalian toxicity and insect deterrency between these compounds and ryanodine suggests a mechanism of action of these diterpenes in insects different from the Ca^{2+} release channel.

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