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LIMONOIDS FROM *SWIETENIA HUMILIS* AND *GUAREA GRANDIFLORA* (MELIACEAE)†

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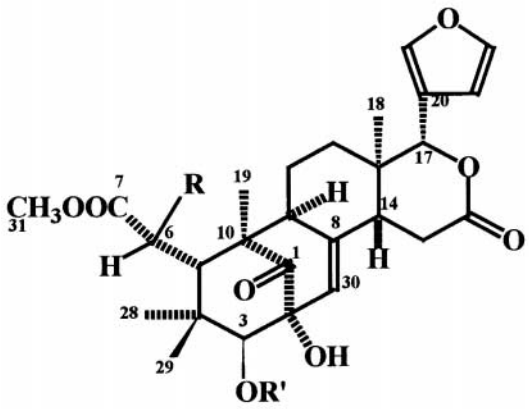
Key Word Index—*Swietenia humilis*; *Guarea grandiflora*; Meliaceae; limonoids; humilinolides A-F; humilin B; swietenin C; methyl-2-hydroxy-3β-isobutyroxy-1-oxomeliac-8(30)-enate; methyl-2-hydroxy-3β-tigloyloxy-1-oxomeliac-8(30)-enate; swietemahonin C; 21α-acetylmelianone; melianone; melianodiol; gedunin; 7-deacetoxy-7-oxogedunin 6-α-acetoxygedunin; insecticides; European corn borer; *Ostrinia nubilalis*.

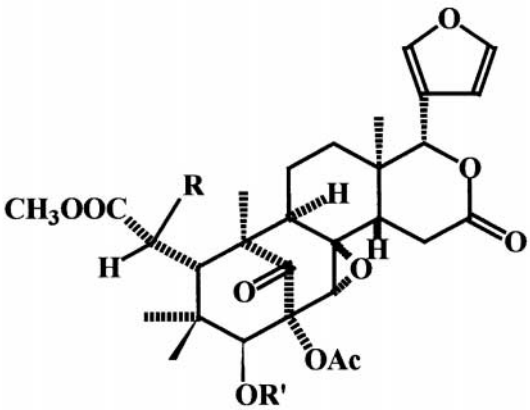
Abstract—Two new limonoids, humilinolides E and F, were isolated from the seeds of *Swietenia humilis* Zucc. (Meliaceae). In addition, the known compounds humilinolides A-D, humilin B, swietenin C, methyl-2-hydroxy-3-β-isobutyroxy-1-oxomeliac-8 (30)-enate, methyl-2-hydroxy-3-β-tigloyloxy-1-oxomeliac-8 (30)-enate and swietemahonin C were isolated for biological evaluation, with swietenin and swietemahonin C reported for the first time from this species. From the seeds of *Guarea grandiflora* A.D.C. (Meliaceae) three protolimonoids, namely 21-α-acetylmelianone, melianone and melianodiol, and three limonoids, gedunin, 7-deacetoxy-7-oxogedunin and 6-α-acetoxygedunin, were obtained. The isolated compounds were identified by spectroscopic methods. Some of the limonoids and protolimonoids were evaluated for their effects on the European corn borer, *Ostrinia nubilalis*, in comparison with toosendanin a commercial insecticide derived from *Melia azederach*.

INTRODUCTION

The Meliaceae family is characterized by the presence of limonoid triterpenes, many of which are biologically active against insects. From the Asian species *Azadirachta indica* and *Melia azederach*, two limonoids have been commercialized: azadarichtin in the U.S. and toosendanin in China. Extracts of several species from the genera *Cedrela*, *Trichilia*, *Swietenia* and *Guarea* from the neotropics have demonstrated moderate to good growth reducing activity to European corn borer (ECB), *Ostrinia nubilalis* Hubner [1–3]. Consequently, some of these species have been the subject of chemical and biological investigations in order to isolate new insecticidal agents. Previously, we have described the isolation and structure elucidation of four novel limonoids from *Swietenia humilis* Zucc., namely humilinolides A–D (**3**–**6**) [4, 5] and a new rearranged limonoid from *Cedrela salvadorensis* Standley, cedrelanolide I [6]. Their effects on the ECB were also evaluated and it was found that the five compounds caused larval mortality, as well as growth reduction and increased the development time of survivors when incorporated into artificial diets of neonates at 50 ppm. The limonoids showed comparable activity to toosendanin (**18**) [7]. In the present study, we have re-investigated the seeds of *S. humilis* and isolated two novel limonoids that were given the trivial names of humilinolides E (**1**) and F (**2**). In addition, the current study of the seeds of *Guarea grandiflora* A.D.C. has led to the isolation of several limonoids and protolimonoids, including the new natural product 21-α-acetylmelianone. In this paper, we describe the structure elucidation and the effects of some of the isolated compounds on the ECB.

*Authors to whom correspondence should be addressed.
†Taken in part from the PhD and MS theses of C. Villarreal and M. A. Jiménez, respectively.





R= OAc, R'= $COC(CH_3)=CHCH_3$ (24, 27, 32) **1**

R= OAc, R'= $COC(CH_3)=CHCH_3$ (24, 27, 32) **2**

R= OH, R'= $COCHCH_3$ (CH_3 at 27) (24, 26) **8**

R= H, R'= $COCHCH_3$ (CH_3 at 27) (24, 26) **7**

R= H, R'= $COCHCH_3$ (CH_3) **9**

R= OAc, R'= $COCHCH_3$ (CH_3) **11**

R= H, R'= $COC(CH_3)=CHCH_3$ (CH_3) **10**

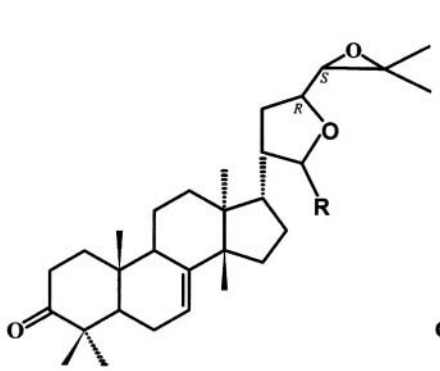
RESULTS AND DISCUSSION

Isolation and characterization of limonoids from S. humilis

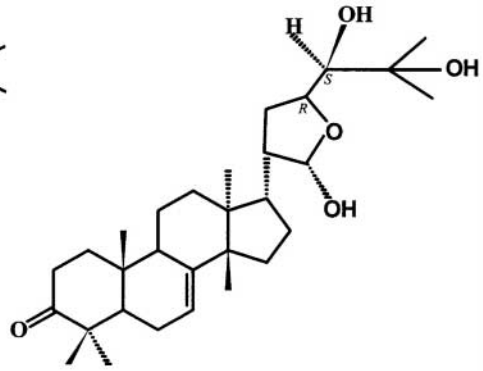
The $CHCl_3$ extract obtained by extracting the defatted seeds of *S. humilis* was separated by a combination of chromatographic procedures to yield two new limonoids, namely, humilinolides E (**1**) and F (**2**). In addition, the known compounds humilinolides A-D (**3**–**6**), humilin B (**7**), swietenin C (**8**), methyl-2-hydroxy-3-β-isobutyroxy-1-oxomeliac-8(30)-enate (**9**), methyl-2-hydroxy-3-β-tigloyloxy-1-oxomeliac-8(30)-enate (**10**) and swietemahonin C (**11**) were isolated. Compounds **8** and **11** are new to this species and their spectral properties, including ^1H and ^{13}C NMR data, were identical to those previously described [8, 9]. Furthermore, the structure of compound **11** was unequivocally assigned as swietemahonin C [9] by X-ray crystallography. The molecular structure of **11** is illustrated in Fig. 1 showing the *syn* relation between the oxo group at C-1 and the oxirane ring and, between the isobutyroxy and acetoxy moieties attached to C-3 and C-6, respectively. According to the Cremer and Pople parameters [10], the conformation of ring A ($q_3 = 0.030$, $Q_T = 0.775$, $\theta = 87.79$, $\phi = 194.93$) is intermediate between boat and twist-boat. Ring B ($q_3 = 0.375$, $Q_T = 0.441$, $\theta = 49.67$, $\phi = 338.96$) possesses a half-chair conformation, while that of ring C ($q_3 = 0.002$, $Q_T = 0.699$, $\theta = 103.53$, $\phi = 89.81$) is between a boat and a twist-boat. Finally, ring D ($q_3 = 0.293$, $Q_T = 0.387$, $\theta = 127.09$, $\phi = 164.16$) exhibits a conformation intermediate between a half-chair and a sofa. The furan ring F is essentially planar.

Compounds **7**, **9** and **10** were previously isolated from the seeds of *S. humilis* [11]. At that time, compounds **9** and **10** were obtained as a mixture that was very difficult to separate and their structures were proposed based on the ^1H NMR and MS analyses of the mixture. In the present study we have separated **9** and **10** by preparative HPLC. In the case of the re-isolation of compound **7**, the relevant ^1H NMR signals match those of humilin B [11]. The identification of compounds **3**–**6** was performed by comparison of their spectral and physical properties with those of authentic samples [4].

The molecular formula of **1**, a colorless crystalline solid, was established as $C_{34}H_{42}O_{11}$ (m/z 626.2635) from the HREIMS. Comparison of the NMR spectra of **1** (Tables 1 and 2) with those of humilinolide D (**6**) [4] revealed that both compounds possessed an identical skeletal structure, varying only in the nature of the acid residues esterifying the hydroxyl group at C-3 of the methyl 2,3-dihydroxy-1-oxomeliacate nucleus. Thus, the acetyl



R= α OAc 12
R= α OH, β OH 13



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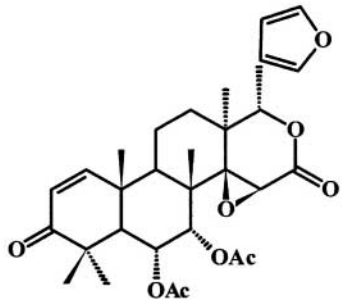

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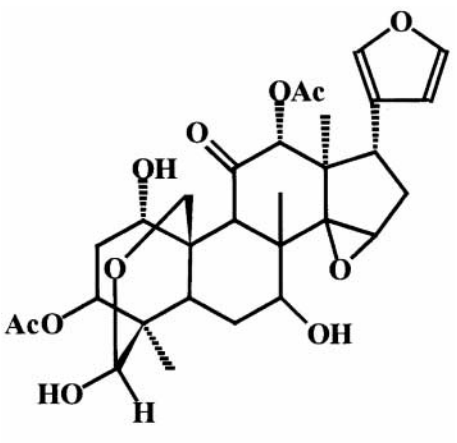
group in **6** was replaced by a tigloyl moiety (δ_H 6.91, 1.82 and 1.74; δ_C 167.1, 127.5, 139.6, 14.6 and 11.8) in compound **1**. The placement of the acetoxy and tigloyloxy groups at C-6 and C-3, respectively, was confirmed by analysis of the HMBC spectrum, which showed the correlations H-6/C-5, C-7, 6-*C*OMe and H-3/C-24, C-28 and C-29. The NOE correlations in the phase-sensitive NOESY spectrum, H-3/H-29, H-28, and H-5, H-5/H-6, H-17, H-26 and H-29, H-19/H-9 and H-6, H-18/H-14 and H-21 and H-26/H-29, H-6, H-5 and H-32 provided evidence for the relative stereochemistry of **1**. Finally, the absolute stereochemistry of compound **1** was determined on the basis of the negative Cotton effect around 300 nm in the CD spectra [8, 9].



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Humilinolide F (**2**), $C_{36}H_{44}O_{12}$, was also obtained as a crystalline compound. The NMR spectra were similar to those of **1**, except that the signals for the 8(30)-trisubstituted olefin and the hydroxyl group at C-2 were missing. In their place were signals for an epoxy moiety (δ_H 3.48; δ_C 62.6 and 65.2) and an additional acetoxy group (δ_H 2.18; δ_C 171.1 and 22.5), respectively. As in the case of compound **1** the allocation of the ester groups and the relative stereochemistry were confirmed by detailed analysis of the HMBC and NOESY spectra, respectively. The NOESY spectrum, in addition to the correlations indicated for compound **1**, showed a strong interaction between H-30 (δ_H3.48) and H-15 (δ_H2.8 and 3.4). This observation supported the α configuration of the epoxy ring. The absolute stereochemistry was determined also considering the negative Cotton effect at 290 nm in the CD spectrum.

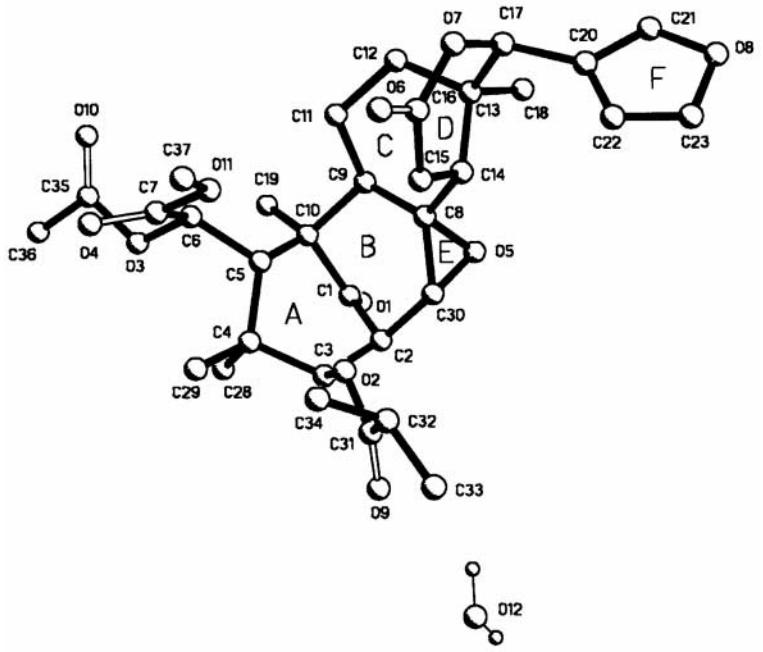

Fig. 1. Stereoscopic view of swietemahonin C.

The NMR (Tables 1 and 2) data of compounds **9** and **10** are in good agreement with the structures initially proposed by Okorie and Taylor [10]. Their structures were fully elucidated following the same strategy as for compounds **1** and **2**.

Isolation and characterization of limonoids and protolimonoids from G. grandiflora

From the seeds of *G. grandiflora* the known limonoids gedunin (**15**), 7-deacetoxy-7-oxogedunin (**16**) and 6α-acetoxygedunin (**17**), were obtained. In addition, three protolimonoids, namely, melianone (**13**), melianodiol (**14**) and 21α-acetylmelianone (**12**) were isolated. Compounds **13**–**17** had been obtained from other Meliaceae species [12–16]. However, this is the first report of the isolation of compound **12** from a natural source. Compound **12** has been previously synthesized from compound **13** (always obtained as the epimeric mixture) on treatment with acetic anhydride and pyridine [14]. The structures of compounds **12**–**17** were readily determined by a combination of spectroscopic analysis and

Table 1. ^{1}H NMR spectral data of compounds **1**, **2**, **9**, and **10** ($CDCl_3$, 500 MHz)*

Proton	**1**	**2**	**9**	**10**
H-3	4.78 *s*	5.72 *s*	4.89 *s*	4.80 *s*
H-5	3.62 *s*	3.45 *s*	3.33 *dd* (9.5, 4.2)	3.39 *dd* (8.1, 3.6)
H-6	5.57 *s*	5.49 *s*	2.37 *dd* (16, 2.5)	2.38 *dd* (17, 2.5)
			2.18 *dd* (16, 9.5)	2.41 *dd* (17, 9)
H-9	2.25 *ddd* (13.8, 3.8, 1.5)	1.95 *m*	2.33 *m*	2.30 *m*
H-15	2.83 *dd* (19.0, 6.0)	2.80 *dd* (17.0, 6.0)	2.85 *dd* (19.0, 6.5)	2.84 *dd* (19.0, 6.0)
	2.90 *dd* (19.0, 1.5)	3.42 *dd* (16.0, 1.3)	2.90 *dd* (19.0, 1.5)	2.91 *dd* (18.0, 2.0)
H-17	5.56 *s*	5.18 *s*	5.68 *s*	5.60 *s*
H-18	1.05 *s*	1.01 *s*	1.23 *s*	1.10 *s*
H-19	1.27 *s*	1.29 *s*	1.11 *s*	1.24 *s*
H-21	7.71 *dd* (1.9, 0.9)	7.46 *dd* (1.8, 0.9)	7.80 *dd* (1.8, 1.0)	7.80 *dd* (1.8, 0.9)
H-22	6.45 *dd* (1.9, 0.9)	6.41 *dd* (1.8, 0.9)	6.47 *dd* (1.9, 1.0)	6.48 *dd* (1.9, 0.9)
H-23	7.44 *dd* (1.9)	7.43 *dd* (1.8)	7.42 *dd* (1.8)	7.43 *dd* (1.8)
H-25	–	–	2.67 *h* (7.0)	–
H-26	6.91 *qq* (7.0, 1.5)	6.98 *qq* (7.5, 1.8)	1.20 *d* (7)	6.81 *qq* (7.2, 1.5)
H-27	1.74 *d* (7.0)	1.94 *d* (7.5)	1.18 *d* (7.0)	1.75 *d* (7.2)
H-28	1.00 *s*	1.18 *s*	0.81 *s*	0.80 *s*
H-29	0.95 *s*	0.92 *s*	0.76 *s*	0.84 s
H-30	5.32 *dd* (1.5)	3.48 *s*	5.38 *dd* (1.8)	5.35 *dd* (1.8)
H-31	3.73 *s*	3.82 *s*	3.71 *s*	3.72 *s*
H-32	1.82 *s*	1.93 *s*	–	1.85 *s*
2-CO*Me*	–	2.18 s	–	–
6-CO*Me*	2.19 *s*	2.19 s	–	–

*Coupling constants (*J*) Hz (in parentheses).

Table 2. ^{13}CNMR spectral data for compounds **1**, **2**, **9** and **10** (125 MHz, $CDCl_3$)*

Carbon	1	2	9	10
C-1	214.0	206.0	215.0	215.0
C-2	77.4	80.8	76.9	76.5
C-3	85.6	85.6	84.6	84.8
C-4	39.8	42.1	39.4	39.5
C-5	45.0	45.1	41.4	41.4
C-6	72.5	72.0	32.7	32.6
C-7	171.0	171.1	173.8	173.9
C-8	136.7	62.6	136.6	136.8
C-9	57.3	55.3	56.6	56.7
C-10	49.5	50.6	49.4	49.3
C-11	21.1	19.9	20.5	20.5
C-12	34.4	33.4	34.4	34.4
C-13	36.7	36.1	36.8	36.9
C-14	44.6	44.9	45.0	45.0
C-15	29.5	33.1	29.8	29.6
C-16	169.7	169.2	169.1	168.6
C-17	77.2	79.5	76.9	76.5
C-18	21.4	26.7	21.9	21.6
C-19	15.5	16.1	15.7	15.7
C-20	120.8	120.2	120.6	120.7
C-21	141.3	140.9	141.9	141.9
C-22	109.4	110.1	109.7	109.7
C-23	143.2	143.3	143.0	143.0
C-24	167.1	166.6	167.6	167.4
C-25	127.5	127.4	34.0	127.5
C-26	139.7	139.7	19.1	139.8
C-27	14.6	14.9	18.6	14.7
C-28	22.3	21.0	19.9	22.1
C-29	22.1	21.3	21.9	19.7
C-30	129.4	65.2	129.0	129.1
C-31	53.3	53.3	52.2	52.2
C-32	11.8	12.6	–	11.9
2-COMe	–	171.1	–	–
2-CO*Me*	–	22.5	–	–
6-COMe	168.28	169.7	–	–
6-CO*Me*	20.93	23.9	–	–

*Assignments were established from HMQC, HMBC and DEPT spectra.

comparison with reported data for these compounds [12–16].

Insecticidal evaluation

Compounds **3**–**6** were evaluated previously [7] and showed comparable effects on the life cycle parameters of ECB as the positive control toosendanin. In the first group of compounds tested, **1**, **9**, **10** and toosendandin (**18**), **10** showed little activity on ECB parameters, but **1** and **9** showed comparable effects to the positive control, toosendandin (**18**), in terms of reduction of % pupation and % adult emergence (Figs 2 and 3). The effects of these compounds on the life cycle parameters of surviving insects (larval weight, time to pupation, pupal weight, time to adult emergence and adult weight) were not significantly different from the control, while toosendanin (**18**) had effects on most of these parameters. In the next group tested (**7** and **8**) only adult emergence was effected (Fig. 4).

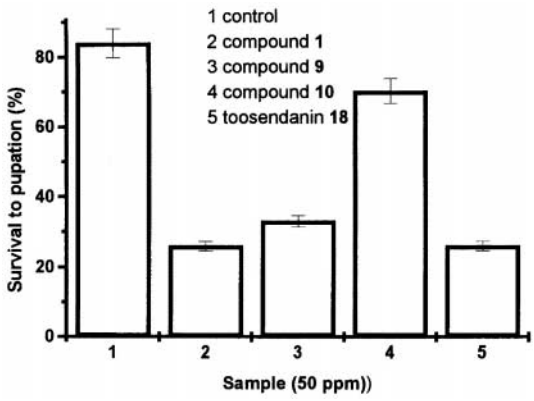

Fig. 3. Effect of compounds **1**, **9** and **10** (50 ppm) on survival to adult stage (%) of ECB.

The compounds available in sufficient quantity for testing from *Guarea grandifolia* were **12**–**14** and **17**. All compounds affected larval growth compared to the control, with **12** and **13** being the most active (Fig. 5). In this case, larval mortality was not affected. However, the % of pupation was significantly reduced by compound **14**. The pupal weight was not affected by any of the compounds, but the time to reach pupation was affected by **12**.

Consequently, we can conclude that **1**, **7**, **8**, **9** and **14** reduced survivorship at various stages while **12**, **14** and **17** reduced growth at the test concentration of 50 ppm. Although of the presently tested compounds were less active than the commercial insecti-

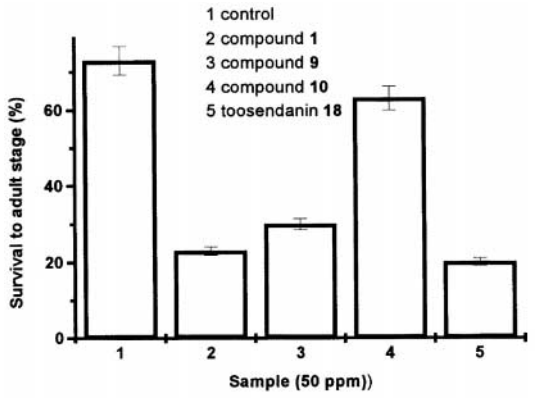

Fig. 2. Effect of compounds **1**, **9** and **10** (50 ppm) on survival to pupation (%) of ECB.

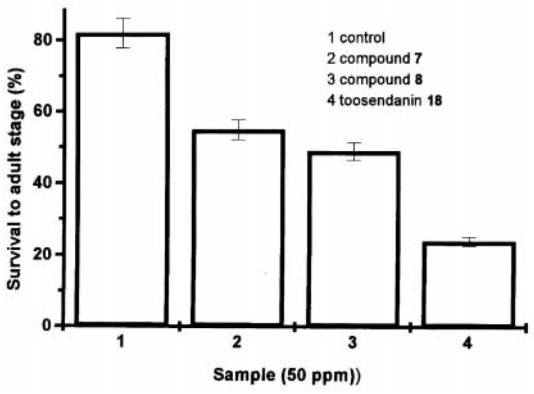

Fig. 4. Effect of compounds **7** and **8** (50 ppm) on survival to adult stage (%) of ECB.

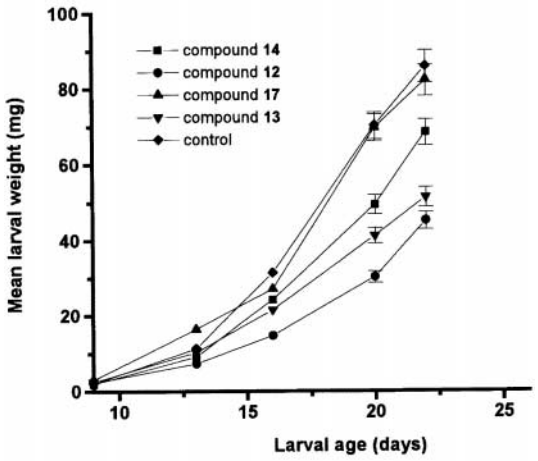

Fig. 5. Growth of ECB larvae fed control diets or diets treated with 50 ppm of compounds **12**–**14** and **17**.

cide toosendanin (**18**), their activity at 50 ppm suggests that the limonoids are potent naturally occurring insect defenses in seeds, where they can be found in concentrations of 73–18 and 900 ppm in *G. grandifolia* and from 290 to 470 ppm in *S. humilis* (Table 3).

Given that compounds **3**–**6** were previously determined [7] to be as active as toosendanin and that the seeds of *S. humilis* now are shown to contain these and other insecticidal compounds in good yield (Table 3), we believe this species is a promising lead for the production of a practical insecticidal standardized extract.

EXPERIMENTAL

General

IR spectra were obtained in KBr on a Perkin-Elmer 599 B spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300 S or on Varian VXR-500 S spectrometers. Melting points were determined in a Fisher Johns apparatus and are uncorrected. Optical rotations were taken on a digital polarimeter JASCO Dip 360. CD spectra were performed on a JASCO 720 spectropolarimeter at 25°C in $CHCl_3$ solutions. EI and HR EI mass spectra were obtained in a JEOL JMS-AX505HA mass spectrometer. Semi-preparative HPLC was performed on a [Nova Pack HR C-18 column (6 μm, 19 id $\times$ 300 mm, Waters)] at a flow rate of 8 ml min^{-1}. The effluent was monitored with a UV detector at 214 nm. Analytical and preparative TLC were performed on precoated silica gel 60 F254 plates (Merck). TLC spots were visualized by spraying with a 10% solution of $Ce(SO_4)_2$ in 2 N H_2SO_4, followed by heating at 110°C. For open CC, silica gel 60 (70–230 mesh, Merck) was used.

Table 3. Estimated concentration of limonoids in *S. humilis** and *G. Grandiflora*** seeds

Compound	Concentration (%)
1*	0.029
2*	0.036
3*	0.290
4*	0.045
5*	0.290
6*	0.054
7*	0.021
8*	0.036
9*	0.032
10*	0.047
11*	0.076
12**	0.0073
13**	0.890
14**	1.89

Plant material

The seeds of *G. grandiflora* were collected in Municipio de San Andrés, Los Tuxtlas, Veracruz, México, in August 1994. A voucher specimen (R. Ortega No. 710) has been deposited in the Herbarium of the Instituto de Ecología (XAL). The seeds of *S. humilis* were collected in the State of Guerrero, Mexico in July 1993. A voucher specimen (Bye and Linares 18824) is deposited in the ethnobotanical collection of the National Herbarium (MEXU), Instituto de Biología, UNAM.

Isolation of limonoids from the seeds of S. humilis

The air-dried plant material (1 kg) was ground into powder and extracted first with hexane (3 l $\times$ 3) at room temperature. The marc was then extracted by maceration with $CHCl_3$ (9 l $\times$ 3). After filtration, the $CHCl_3$ extract was concentrated *in vacuo* to yield a yellow oil (125 g). This oil was subjected to column chromatography (CC) over Si-gel (1.25 kg) using a gradient of increasing polarity with benzene–EtOAc–MeOH to yield three primary fractions (SI–SIII). Fraction SII (22.8 g), eluted with benzene–EtOAc (3:7), was rechromatographed on a silica gel column and eluted with a solvent gradient system from hexane to EtOAc and from EtOAc to MeOH. A total of eight secondary fractions were obtained (SIIA–SIIH). From fraction SII-E crystallized spontaneously **5** (220 mg), m.p. 210–213°C. From fraction SIIG **4** (40 mg), m.p. 280–282°C, were isolated. Fraction SIII (31.4 g) was rechromatographed on silica gel (628 g), using the same elution system as for fraction S-II. Ten secondary fractions were obtained (SIIIA–SIIIJ). Extensive TLC [benzene–EtOAc (8:2)] of fraction SIIIF yielded **11** (38.5 mg), m.p. 310–311°C [lit mp 309–310°C (9)]. From fraction SIIIG co-crystallized a mixture (277 mg) of **3** and **5**. The mixture was resolved by column chromatography on silica gel, eluting with a mixture of benzene–EtOAc (8:2), to yield **3** (254 mg), m.p. 256–258°C, and **5** (21.4 mg), m.p. 262–264°C. The mother liquors from fraction SIIIG (9.5 g) were re-chromatographed on a column packed with silica gel (190 g). The elution was

accomplished with benzene with increasing amounts of EtOAc. This chromatographic process afforded 13 fractions (SIIIG1–SIIIG13). HPLC purification of fraction SIIIG6 on a reversed phase silica gel column [8 ml min^{-1}; CH_3CN–H_2O (55:45)] yielded **8** (23 mg), m.p. 239–240°C [lit m.p. 238–239°C (8)], **7** (13.8 mg), m.p. 212–213°C and **5** (17 mg). The retention times were, 21, 25 and 62.5 min, respectively. HPLC separation of fraction SIIIG12 [8 ml min^{-1}; CH_3CN–H_2O (50:50)] afforded additional amounts of **3** (17 mg, $R_t = 18$ min) and **5** (13 mg, $R_t = 19.5$ min), **9** (20.7 mg, $R_t = 26.5$ min), **1** (19 mg, $R_t = 33$ min) and **10** (30 mg, $R_t = 37$ min). Finally, extensive HPLC separation of fraction SIIIG13, using the same conditions as for fraction SGIII12, afforded **2** (18 mg, $R_t = 45$ min). (lit m.p. 179°C [32]).

*Humilinolide E (**1**)*

Crystalline needles, m.p. 98–100°C. $[\alpha]_D$ −31.5 ($CHCl_3$). CD ($CHCl_3$) $\Delta\varepsilon$ (nm): -2.63×10^3 (293.5). IR ν_{max} (KBr) cm^{-1}: 3453, 1730, 1668, 1223, 1127, 873 and 755. ^1H and ^{13}C NMR (Tables 1 and 2, respectively). EM EI *m*/*z* (rel int): 626 [M^+ (44)], 568 (6), 543 (3), 526 (7), 483 (12), 469 (14), 395 (10), 269 (5), 191 (20), 134 (29), 96 (21), 83 (100), 54 (43). HREI MS *m*/*z*: Found 626.2635 (calcd 626.2726) for $C_{34}H_{42}O_{11}$.

*Humilinolide F (**2**)*

Crystalline needles, m.p. 112–115°C. $[\alpha]_D$ −41.4 ($CHCl_3$). CD ($CHCl_3$) $\Delta\varepsilon$ (nm): -14.06×10^3 (290.5) IR ν_{max} (KBr) cm^{-1}: 1756–1744, 1710, 1233, 1127, 873 and 733. ^1H and ^{13}C NMR (Tables 1 and 2, respectively). EM EI *m*/*z* (rel int): 684 [M^+ (31)], 624 (7), 585 (23), 553 (4), 546 (5), 525 (15), 437 (12), 377 (5), 295 (8), 235 (14), 95 (24), 83 (100), 55 (40).

*Methyl-2-hydroxy-3β-isobutyroxy-1-oxomeliac-8(30)-enate (**9**)*

Crystalline needles, m.p. 89–90°C. $[\alpha]_D$ −33.9 ($CHCl_3$). CD ($CHCl_3$) $\Delta\varepsilon$ (nm): -8.06×10^3 (294). IR ν_{max} (KBr) cm^{-1}: 3459, 1726, 1710, 1224, 1133, 1025 and 758. ^1H and ^{13}C NMR (Tables 1 and 2, respectively). EM EI *m*/*z* (rel int): 556 [M^+ (100)], 486 (8), 453 (9), 379 (54), 367 (10), 269 (12), 196 (14), 134 (39), (14), 95 (52), 83 (61).

*Methyl-2-hydroxy-3β-tigloyloxy-1-oxomeliac-8(30)-enate (**10**)*

Crystalline needles, m.p. 100–102°C. $[\alpha]_D$ −15.9 ($CHCl_3$). CD ($CHCl_3$) $\Delta\varepsilon$ (nm): -2.55×10^3 (294). IR ν_{max} (KBr) cm^{-1}: 3443, 1732 (*br*), 1233, 1158, 853 and 755. ^1H and ^{13}C NMR (Tables 1 and 2, respectively). EM EI *m*/*z* (rel int): 568 [M^+ (50)], 550 (4), 486 (6), 453 (14), 435 (3), 395 (19), 296 (5), 191 (7), 173 (5), 295 (8), 95 (20), 83 (100).

Isolation of limonoids and protolimonoids from the seeds of G. grandiflora

The air dried and pulverized seeds of *G. grandiflora* (637 g) were extracted with hexane–$CHCl_3$ (1:1) (3 l × 3) by cold maceration. The resulting extract was concentrated *in vacuo* to afford a yellow residue (73 g). Open column chromatography of portion of the extract (69 g) was performed on silica gel (690 g) eluted with hexane, hexane–$CHCl_3$ (different proportions), $CHCl_3$–EtOAc (different proportions) and then EtOAc to yield nine major fractions (GI–GIX). TLC [$CHCl_3$–EtOAc (95:5)] purification of fraction SII (0.91 g), eluted with hexane–$CHCl_3$ (1:1), yielded **12** (39 mg) as colorless oil. From fraction SIV, eluted with $CHCl_3$, crystallized **15** (67 mg), m.p. 217–218°C, identical to an authentic sample. From fraction SVI, eluted with $CHCl_3$–EtOAc (7:3), co-crystallized a binary mixture (3.5 g) of **16** and **17**. Extensive TLC [benzene–EtOAc (3:1) separation of the mixture afforded **17** (2.7 g), m.p. 275°C [lit m.p. 270–273°C (13)] and **16** (500 mg), m.p. 262–265°C, identical to an standard sample. Fraction SVII, eluted with $CHCl_3$–EtOAc (1:1), yielded **13** (1.8 g), m.p. 230°C [lit m.p. 232–233°C (16)]. Finally, from fraction SVIII, eluted with $CHCl_3$–EtOAc (2:8), **14** (1.21 g), m.p. 220°C [lit m.p. 219–222°C (15)], was obtained.

X-ray crystallography

Swietemahonin C hemihydrate gave crystals from MeOH: $C_{33}H_{42}O_{11}$ 0.5 H_2O, orthorhombic, $V = 3275.1(5)$ Å^3, space group $P2_12_12_1$ (No. 19) with cell constants $a = 12.194(1)$ Å, $b = 12.559(1)$ Å, $c = 21.386(2)$ Å, $D_c = 1.265$ g cm^{-3} and 4 molecules in the unit cell. The data collection was performed using monochromated CuKα radiation 1.54178 Å. The structure was solved by direct methods (SIR92) [17]. Refinement (SHELXL-97) [18] by least-squares with anisotropic displacement parameters for all non-hydrogen atoms resulted in $R = 0.0651$, $wR = 0.1622$. A weighting scheme according to $w = 1/\sigma^2(F_o^2) + (0.0923*P)^2 + 1.03*P]$ where $P = (\max(F_o^2, 0) + 2*F_c^2)/3$.

Hydrogen atoms were treated isotropically in a riding model and only those at O were refined restrained. A total of 413 parameters were considered in the full least-squares refinement using 3248 reflections (*hkl* plus Friedel pairs, $2\theta \leq 113.5°$). The absolute configuration could not be determined; relative orientation at the asymmetric carbons as given in Fig. 1. Listing of positional and displacement parameters, tables of bond distances and angles have been submitted as supplementary material to be deposited in the Cambridge Crystallographic Data Center.

Bioassays with ECB

Larvae used for the experiments were obtained from the culture at the University of Ottawa, which

was maintained under previously described conditions. All test materials were dissolved in 95% ethanol and added to the artificial diet at one concentration (50 ppm) or control (1 ml 95% ethanol). Neonate larvae were placed collectively for 9 days in glass vials containing a cube of the appropriately treated diet. Thirty larvae were then transferred to separate vials containing the corresponding diet cubes. Larvae were weighed approximately every four days, at which time the old diet was replaced with fresh stock. Larval weight gains and mortality were recorded the last day before the first larva pupated (approximately after 20 days). Other life-cycle measurements were recorded, such as time to pupation and adulthood, weight of pupae and adults, mortality of larvae and adult deformities. All treatments were effected in a controlled environment chamber with an 18L:6D photoperiod, a 25°C day and 19°C night temperature regime, and a relative humidity of approximately 80%. Data analyses for all the live insect bioassays were statistically analyzed using SAS ANOVA and GLM procedures.

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