Insect Antifeedant Activity of Three New Tetranortriterpenoids from *Trichilia pallida*

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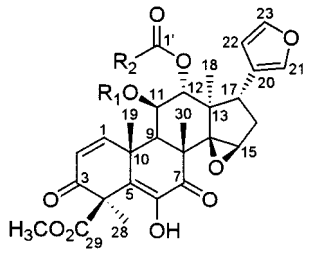
Three new tetranortriterpenoids, methyl 6-hydroxy-11β-acetoxy-12α-(2-methylpropanoyloxy)-3,7-dioxo-14β,15β-epoxy-1,5-meliacadien-29-oate (**3**), methyl 6,11β-dihydroxy-12α-(2-methylpropanoyloxy)-3,7-dioxo-14β,15β-epoxy-1,5-meliacadien-29-oate (**4**), and methyl 6-hydroxy-11β-acetoxy-12α-(2-methylbutanoyloxy)-3,7-dioxo-14β,15β-epoxy-1,5-meliacadien-29-oate (**5**), have been isolated from the roots of *Trichilia pallida*. The related compounds hirtin (**1**) and deacetylhirtin (**2**) were also obtained. Compound **4** had the greatest antifeedant activity of **1**−**5** when tested against larvae of four species of Lepidoptera.

Extracts of the genus *Trichilia* L. (Meliaceae) are reported to have a variety of biological properties, including antiviral,[1] analgesic,[2] insecticidal,[3] and insect growth-inhibition activity.[4] Their anti-insect activity has been attributed to a group of tetranortriterpenoids that includes hirtin[4] and the trichilins.[3,5] The current report describes the isolation and structural elucidation of three new tetranortriterpenoids from *Trichilia pallida* Sw. and two related compounds, hirtin (**1**) and deacetylhirtin (**2**). A binary-choice bioassay was used to test the antifeedant activity of the compounds against four species of Lepidoptera: *Spodoptera littoralis*, *S. exigua*, *Heliothis virescens*, and *Helicoverpa armigera*.

An acetone extract of the roots of a five-year-old *T. pallida* tree grown under glasshouse conditions was analyzed by HPLC coupled to a photodiode-array detector. Five major apolar components (**1**−**5**) were detected with UV−vis spectra similar to that of the tetranortriterpenoid limonin,[6] which shows distinctive UV maxima at 217 and 276 nm. Milligram quantities of **1**−**5** sufficient for structure elucidation were obtained only by repetitive isolation using an analytical HPLC column, because of the loss of resolution experienced on semipreparative columns.

The ^{1}H and ^{13}C NMR spectra of **1** recorded in $CDCl_3$ contained a number of resonances typical of tetranortriterpenoids including four quaternary methyl groups (δ_H 0.79, 1.40, 1.43 and 1.82), a methyl ester (δ_H 3.76; δ_C 53.0 and 170.0), and a β-substituted furan (δ_H 6.08, 7.11, and 7.30; δ_C 111.1, 121.7, 140.5, and 142.7). Many examples of this class of compound have been reported previously from *Trichilia* spp.[7] Other distinctive resonances included those for an acetyl group (δ_H 2.19; δ_C 21.1 and 169.4), a propanoyl side chain (δ_H 1.02, 2.19, and 2.27; δ_C 8.9, 27.7, and 172.2), and two α,β-unsaturated carbonyls (δ_H 7.01 and 6.16; δ_C 150.7, 126.8, and 195.6; δ_C 129.4, 141.9, and 196.2) located at C1−C3 and C5−C7, respectively, according to HMBC correlations. A molecular formula of $C_{32}H_{36}O_{11}$ was obtained for **1** by HRMS. Analysis of both 1D and 2D NMR data (1D ^{1}H and ^{13}C, DEPT, DQF-COSY, HSQC, and HMBC) confirmed the molecular structure of **1** to be that of hirtin, a known tetranortriterpenoid isolated previously from *T. hirta*.[8] Resonance assignments are summarized in Tables 1 and 2, and long-range correlations obtained from HMBC data are listed in Table 1S (Supporting Information). The ^{1}H NMR assignments for **1** are in agreement with a partial set available from the literature,[8] but ^{13}C NMR assignments for this compound have not been reported previously (Table 2).



	R_1	R_2
1	Ac	CH_2CH_3
2	H	CH_2CH_3
3	Ac	$CH(CH_3)CH_3$
4	H	$CH(CH_3)CH_3$
5	Ac	$CH(CH_3)CH_2CH_3$

The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, the only significant difference being the absence of resonances corresponding to the 11-OAc group of **1** (Tables 1 and 2). The chemical shift value of H-11 in **2** (δ_H 4.21) was shifted upfield compared to that of **1**, consistent with the absence of the acetyl group. HRMS of **2** gave a molecular formula of $C_{30}H_{34}O_{10}$ (42 amu less than **1**) and confirmed its identity as deacetylhirtin, a compound also known from *T. hirta*.[8] A full set of ^{1}H and ^{13}C NMR resonance assignments for **2** are presented in Tables 1 and 2, respectively.

The NMR spectra of **3**−**5** were also similar to those of **1** but contained additional resonances in the aliphatic regions. Two of the compounds (**3** and **5**) were found to be acetylated at C-11, as in **1**. Comparison of the spectra of **3** and **1** indicated that the propanoyl side chain at C-12 of the latter was replaced by a 2-methylpropanoyl side chain (Tables 1 and 2). The two methyl resonances of **3** at δ_H 0.97 (d, J = 7.1 Hz) and δ_H 1.07 (d, J = 7.1 Hz) showed cross-peaks in the DQF-COSY spectra to the septet at δ_H 2.44,

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Table 1. ^{1}H NMR Chemical Shift Assignments (δ) and Coupling Constant Data for Compounds **1**−**5** in $CDCl_3$ at 30 °C

proton	**1**	**2**	**3**	**4**	**5**
1	7.01 (d, 10.2)	6.91 (d, 10.3)	6.97 (d, 10.2)	6.87 (d, 10.2)	6.96 (d, 10.3)
2	6.16 (d, 10.1)	6.13 (d, 10.3)	6.15 (d, 10.1)	6.12 (d, 10.2)	6.14 (d, 10.2)
9	2.97 (s)	2.71 (br s)	2.98 (s)	2.69 (s)	2.97 (s)
11	5.39 (br s)	4.21 (br s)	5.35 (br s)	4.16 (br s)	5.36 (br s)
12	5.22 (br s)	5.09 (br s)	5.20 (br s)	5.10 (br s)	5.22 (br s)
15	3.91 (s)	3.98 (s)	3.91 (s)	3.98 (s)	3.90 (s)
16	2.00 (dd, 13.7, 11.1)	1.98 (ddd, 13.9, 11.1, 0.7)	1.99 (dd, 13.9, 11.1)	2.01 (dd, 13.8, 11.1)	1.99 (ddd, 13.9, 11.1, 0.7)
	2.31 (ddd, 13.7, 6.7, 0.6)	2.34 (ddd, 13.9, 6.6, 0.7)	2.32 (ddd, 13.9, 6.6, 0.5)	2.34 (dd, 13.8, 6.7)	2.31 (ddd, 13.9, 6.9, 0.7)
17	2.92 (dd, 11.0, 6.8)	2.92 (dd, 11.0, 6.6)	2.93 (dd, 11.1, 6.8)	2.94 (dd, 11.0, 6.7)	2.94 (dd, 11.1, 6.9)
18	0.79 (s)	0.75 (s)	0.81 (s)	0.77 (s)	0.81 (s)
19	1.43 (s)	1.67 (s)	1.43 (s)	1.66 (s)	1.43 (s)
21	7.11 (m)	7.13 (m)	7.12 (m)	7.13 (m)	7.13 (m)
22	6.08 (dd, 1.6, 0.7)	6.06 (dd, 1.7, 0.7)	6.10 (m)	6.08 (dd, 1.6, 0.7)	6.10 (dd, 1.7, 0.7)
23	7.30 (t, 1.6)	7.32 (t, 1.7)	7.29 (t, 1.7)	7.32 (t, 1.6)	7.28 (t, 1.6)
28	1.82 (s)	1.83 (s)	1.83 (s)	1.83 (s)	1.83 (s)
30	1.40 (s)	1.41 (s)	1.40 (s)	1.41 (s)	1.40 (s)
2′	2.19 (m)	2.27 (m)	2.44 (septet, 7.1)	2.48 (septet, 7.0)	2.26 (sextet, 7.0)
	2.27 (dq, 16.3, 7.6)				
3′	1.02 (t, 7.6)	1.07 (t, 7.6)	1.07 (d, 7.1)[a]	1.08 (d, 7.0)[a]	0.96 (d, 7.0)
4′			0.97 (d, 7.1)[a]	1.03 (d, 7.0)[a]	1.42 (m)
					1.62 (m)
5′					0.88 (t, 7.5)
6-OH	6.41 (s)	6.39 (s)	6.41 (s)	6.39 (s)	6.41 (s)
11-OAc	2.19 (s)		2.18 (s)		2.18 (s)
CO_2CH_3	3.76 (s)	3.76 (s)	3.76 (s)	3.76 (s)	3.76 (s)

[a] Assignments may be interchanged.

Table 2. ^{13}C NMR Chemical Shift Assignments (δ) for Compounds **1**−**5** in $CDCl_3$ at 30 °C

carbon	**1**	**2**	**3**	**5**
1	150.7	151.4	150.5	150.5
2	126.8	126.5	126.9	126.8
3	195.6	195.7	195.5	195.5
4	59.3	59.3	59.3	59.3
5	129.4	129.6	129.3	129.3
6	141.9	141.9	141.9	142.0
7	196.2	196.5	196.2	196.3
8	46.2	45.9	46.2	46.2
9	42.9	43.9	42.9	42.8
10	39.9	40.5	39.9	40.0
11	72.5	73.2	72.4	72.4
12	78.5	82.8	77.9	77.9
13	45.1	44.8	45.3	45.3
14	67.7	68.3	67.6	67.6
15	55.1	56.5	55.1	55.1
16	32.1	31.6	32.2	32.3
17	41.6	42.2	41.5	41.5
18	15.6	15.3	15.6	15.7
19	25.4	26.5	25.3	25.2
20	121.7	121.9	121.5	121.5
21	140.5	140.1	140.5	140.6
22	111.1	111.0	111.2	111.5
23	142.7	142.9	142.7	142.6
28	22.9	22.8	22.9	22.9
29	170.0	170.2	170.0	170.0
30	22.5	22.5	22.5	22.5
1′	172.2	173.9	174.4	174.2
2′	27.7	27.8	34.1	41.0
3′	8.9	9.0	18.6[a]	16.3
4′			18.7[a]	26.6
5′				11.5
$OCOCH_3$	169.4		169.3	169.3
$OCOCH_3$	21.1		21.1	21.1
CO_2CH_3	53.0	52.9	53.0	53.0

[a] Assignments may be interchanged.

and long-range correlations in HMBC spectra to the carbonyl at δ_C 174.4 (C-1′). An HMBC connectivity from H-12 (δ_H 5.20) to the same carbonyl confirmed that the 2-methylpropanoyl side chain of **3** was attached at C-12. HRMS of **3** gave a molecular formula of $C_{33}H_{38}O_{11}$ and was consistent with the presence of the larger side chain at C-12. Compound **3** was therefore identified as methyl 6-hydroxy-11β-acetoxy-12α-(2-methylpropanoyloxy)-3,7-dioxo-14β,15β-epoxy-1,5-meliacadien-29-oate, a new derivative of hirtin.

Compound **4** was the least abundant of the tetranortriterpenoids isolated from *T. pallida*, and only material sufficient for ^{1}H NMR analysis was obtained (<0.3 mg). Analysis of the ^{1}H NMR spectrum of **4** revealed close similarities to that of **3** and indicated that **4** also contained a 2-methypropanoyl side chain at C-12 (Table 1). However, **4** lacked the characteristic resonance corresponding to the acetyl group of **3** (δ_H 2.18), and its H-11 resonance (δ_H 4.16) was shifted upfield by −1.19 ppm (Table 1). HRMS of **4** gave a molecular formula of $C_{31}H_{36}O_{10}$, 42 amu less than that of **3**. These data indicated that **4** was methyl 6,11β-dihydroxy-12α-(2-methylpropanoyloxy)-3,7-dioxo-14β,15β-epoxy-1,5-meliacadien-29-oate, the 11-deacetyl derivative of **3** and another new derivative of hirtin.

The NMR spectra of **5** were also similar to those of **3** with the exception of some differences in the aliphatic region. A terminal methyl group (3H, δ_H 0.88, t, J = 7.5 Hz) was found to be connected to a CH_2 group adjacent to a $CH(CH_3)$ fragment from the DQF-COSY data. Long-range connectivities from the CH resonance (δ_H 2.26) and H-12 (δ_H 5.22) to the carbonyl at δ_C 174.2 were also detected. These confirmed the presence of a 2-methylbutanoyl side chain at C-12 as the only difference between **5** and **3**, the latter having a 2-methylpropanoyl side chain at the same position. HRMS of **5** gave the molecular formula $C_{34}H_{40}O_{11}$, which was 14 amu greater than **3**, as expected. Compound **5** was therefore confirmed to be methyl 6-hydroxy-11β-acetoxy-12α-(2-methylbutanoyloxy)-3,7-dioxo-14β,15β-epoxy-1,5-meliacadien-29-oate, a third new derivative of hirtin.

Compound **4** was the only one of the five tetranortriterpenoids to deter the larvae of all four species of Lepidoptera from feeding (Table 3). Although hirtin (**1**) had previously been shown to inhibit feeding and growth of fourth stadium larvae of *Peridroma saucia*, when incorporated into an artificial diet,[4] it showed no activity in our glass-fiber disk bioassay. Similarly, neither **3** nor **5** showed any antifeedant activity. The responses of the species of Lepidoptera to compound **2** varied; it deterred *H. virescens*

Table 3. Effect of the Acetone Extract and Compounds Isolated from *Trichilia pallida* on the Feeding of Final Stadium Larvae of Four Species of Lepidoptera

	feeding index[a] (mean ± sem)[b]			
sample tested	*Spodoptera littoralis*	*Spodoptera exigua*	*Heliothis virescens*	*Helicoverpa armigera*
extract	93 ± 3.6**	nt	nt	nt
1	4 ± 17.6	10 ± 12.8	9 ± 14.8	12 ± 13.9
2	−14 ± 14.1	12 ± 13.9	29 ± 13.6*	32 ± 11.2*
3	5 ± 21.5	7 ± 22.8	8 ± 10.2	16 ± 18.3
4	46 ± 12.3*	40 ± 9.3*	49 ± 15.3*	42 ± 9.9*
5	−17 ± 18.2	6 ± 21.7	4 ± 16.8	−6 ± 23.8

[a] Feeding index = $((C - T)/(C + T)) \times 100$; extract and compounds tested at 100 ppm; nt = not tested. ** $P < 0.01$, * $P < 0.05$, Wilcoxon matched-pairs test. [b] Number of replicates = 10.

and *H. armigera* from feeding on the treated glass-fiber disks but not *S. littoralis* or *S. exigua*. Thus both compounds (**2** and **4**) with a hydroxyl moiety rather than an acetyl moiety at C-11 showed antifeedant activity against *H. virescens* and *H. armigera*. These results support data from earlier research on derivatives of azadirachtin and other related limonoids that showed that small changes to the structure of the molecule, especially the nature of the side chain at C-11, alter its antifeedant activity.[9]

The crude acetone extract of *T. pallida* had potent antifeedant activity against larvae of *S. littoralis* at 100 ppm. However, the antifeedant response of *S. littoralis* to each of the isolated tetranortriterpenoids was lower than that of the crude extract. This would suggest that further experiments should be undertaken to investigate whether there might be additive or synergistic interactions among the tetranortriterpenoids that enhance their antifeedant activity.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were acquired on either Varian 500 MHz or Bruker 400 MHz instruments. All chemical shift values (δ) are given in ppm. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 7.25/77.0 ($CDCl_3$) relative to TMS. Negative ion first-order MS were recorded using LC-MS (Finnigan-Matt LCQ) with an electrospray ionization (ESI) source. HREIMS were determined using a Micromass LC TOF mass spectrometer calibrated with a PEG ammonium calibration solution. HPLC was carried out using a Waters system consisting of a 600E pump, 717 autosampler, and 996-photodiode-array detector.

Plant Material. Root material of *Trichilia pallida* Sw. (Royal Botanic Gardens, Kew accession no. 1992-2045) was collected in 1997 from a five-year-old tree growing in a glasshouse at RBG Kew.

Extraction and Isolation. Roots of *T. pallida* (350 g) were ground to a fine powder and extracted with hexane at room temperature for 24 h. After filtration, the residual plant material was re-extracted with acetone at room temperature for a further 24 h. This extract was filtered and the solvent removed under reduced pressure. The residue was redissolved in acetone and analyzed by HPLC (Merck LiChrospher, 250 × 4.0 mm, 5 μm particle size) at 1 mL/min flow rate, using a solvent gradient of MeOH−MeCN−H_2O, 25:25:50, to MeOH−MeCN−H_2O, 90:10:00, over 20 min. Compounds **1**−**5**, the major apolar components of the acetone extract, were detected at 210 nm and eluted at 18.7 (**2**), 19.8 (**1**), 20.5 (**4**), 21.4 (**3**), and 22.9 (**5**) min, respectively. They were isolated by repeated collections from an analytical column to give final yields of **1** (7.4 mg), **2** (3.8 mg), **3** (4.5 mg), **4** (0.3 mg), and **5** (2.3 mg).

Methyl 6-hydroxy-11*β*-acetoxy-12α-propanoyloxy-3,7-dioxo-14*β*,15*β*-epoxy-1,5-meliacadien-29-oate (1) (hirtin): white solid (MeOH); UV (MeOH−H_2O) λ_{max} 217, 276 nm; ^{1}H NMR data, see Table 1, Table 1S (HMBC correlations); ^{13}C NMR data, see Table 2; ESIMS *m/z* 595 $[M - H]^+$; HREIMS *m/z* 597.2319 $[M + H]^+$ (calcd for $C_{32}H_{37}O_{11}$, 597.2336).

Methyl 6,11*β*-dihydroxy-12α-propanoyloxy)-3,7-dioxo-14*β*,15*β*-epoxy-1,5-meliacadien-29-oate (2) (deacetylhirtin): white solid (MeOH); UV (MeOH−H_2O) λ_{max} 217, 276 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; ESIMS *m/z* 553 $[M - H]^+$; HREIMS *m/z* 555.2222 $[M + H]^+$ (calcd for $C_{30}H_{35}O_{10}$, 555.2230).

Methyl 6-hydroxy-11*β*-acetoxy-12 meliacadien-29-oate-(2-methylpropanoyloxy)-3,7-dioxo-14*β*,15*β*-epoxy-1,5-meliacadien-29-oate (3): white solid (MeOH); UV (MeOH−H_2O) λ_{max} 217, 276 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; ESIMS *m/z* 609 $[M - H]^+$; HREIMS *m/z* 611.2473 $[M + H]^+$ (calcd for $C_{33}H_{38}O_{11}$, 611.2492).

Methyl 6,11*β*-dihydroxy-12α-(2-methylpropanoyloxy)-3,7-dioxo-14*β*,15*β*-epoxy-1,5-meliacadien-29-oate (4): white solid (MeOH); UV (MeOH−H_2O) λ_{max} 217, 276 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; ESIMS *m/z* 567 $[M - H]^+$; HREIMS *m/z* 569.2402 $[M + H]^+$ (calcd for $C_{31}H_{37}O_{10}$, 569.2387).

Methyl 6-hydroxy-11*β*-acetoxy-12α-(2-methylbutanoyloxy)-3,7-dioxo-14*β*,15*β*-epoxy-1,5-meliacadien-29-oate (5): white solid (MeOH); UV (MeOH−H_2O) λ_{max} 217, 276 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; ESIMS *m/z* 623 $[M - H]^+$; HREIMS *m/z* 625.2650 $[M + H]^+$ (calcd for $C_{34}H_{41}O_{11}$, 625.2649).

Biological Assay. The compounds were dissolved in acetone and applied to glass-fiber disks in a binary choice test as described previously.[9] The compounds were tested against final stadium larvae, 36−48 h into the stadium, that had been deprived of food for 2−3 h prior to the bioassay. The crude acetone extract of *T. pallida* and the five compounds were tested at 100 ppm. The feeding index $((C - T)/(C + T)) \times 100$ was calculated using the amount of control (C) and treatment (T) disks eaten during the 18 h bioassay. The Wilcoxon matched-pairs test was used to evaluate the significance of the amount of the C and T disks eaten.[10]

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Supporting Information Available: HMBC correlations for **1** in $CDCl_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Roming, T. L.; Weber, B. K.; Murray, B. K.; North, J. A.; Wood, S. G.; Hughes, B. G.; Cates, R. G. *Phytother. Res.* **1992**, *6*, 38−43.
(2) (a) Vaz, Z. R.; Mata, L. V.; Calixto, J. B. *Phytother. Res.* **1997**, *11*, 101−106. (b) Arenas, C.; Rodriguez-Hahn, L. *Phytochemistry* **1990**, *29*, 2953−2956.
(3) Champagne, D. E.; Isman, M. B.; Tower, G. H. N. In *Insecticides of Plant Origin*; Arnason, J. T., Philogene, B. J. R., Morand, P., Eds.; American Chemical Society: Washington, DC, 1989; Chapter 8, pp 95−109.
(4) Xie, Y. S.; Isman, M. B.; Gunning, P.; Mackinnon, S.; Arnason, J. T.; Taylor, D. R.; Sanchez, P.; Hasbunt, C.; Towers, G. H. N. *Biochem. Syst. Ecol.* **1994**, *22*, 129−136.
(5) (a) Nakatani, M. In *Bioactive Compounds from Natural Sources*; Tringali, C., Ed.; Taylor & Francis: London, U.K., 2001; Chapter 13, pp 527−554. (b) Zhou, J.-B.; Okamura, H.; Iwagawa, T.; Nakatani, M. *Phytochemistry* **1995**, *41*, 117−120.
(6) Baldi, A.; Rosen, R. T.; Fukuda, E. K.; Ho, C.-T. *J. Chromatogr. A* **1995**, *718*, 89−97.
(7) (a) Cortez, D. A. G.; Vieira, P. C.; Fernades, J. B.; Da Silva, M. F. G. F.; Fereira, A. G. *Phytochemistry* **1992**, *31*, 625−628. (b) Garcez, F.

R.; Garcez, W. S.; Rodrigues, E. D.; Pott, V. J.; Roque, N. F. *Phytochemistry* **1996**, *42*, 795−798. (c) Tinto, W. F.; Jagessar, P. K.; Ketwaru, P.; Reynolds, W. F.; McLean, S. *J. Nat. Prod.* **1991**, *54*, 972−977. (d) Rodriguez-Hahn, L.; Cardenas, J.; Arenas, C. *Phytochemistry* **1996**, *43*, 457−459. (e) Musza, L. L.; Killar, L. M.; Speight, P.; Barrow, C. J.; Gillum, A. M.; Cooper, R. *Phytochemistry* **1995**, *39*, 621−624.

(8) Chan, W.; Taylor, D. R. *J. Chem. Soc., Chem. Commun.* **1966**, 206−207.

(9) Simmonds, M. S. J.; Blaney, W. M.; Ley, S. V.; Anderson, J. C.; Banteli, R.; Denholm, A. A.; Green, P. C. W.; Grossman, R. B.; Gutteridge, C.; Jennens, L.; Smith, S. C.; Toogood, P. L.; Wood, A. *Entomol. Exp. Appl.* **1995**, *77*, 69−80.

(10) Siegel, S.; Castellan, N. J. *Nonparametric Statistics for the Behavioral Sciences*; McGraw-Hill: New York, 1988.

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