New Limonoids from *Melia toosendan*

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The root bark of *Melia toosendan* afforded four new limonoids with a C-19/C-29 bridged acetal structure, together with two known limonoids, 12 α -hydroxyamoorastatone and its 12-acetate. The new compounds were elucidated as 1-*O*-acetyltrichilin H and 29-*O*-substituted amoorastatone derivatives, named neoazedarachins A, B and D, by spectroscopic and chemical means. Their antifeedant activity was also studied.

Key words: limonoid; antifeedant; *Melia toosendan*; 1-*O*-acetyltrichilin H; neoazedarachins

The limonoids from Meliaceae plants are attracting considerable interest, because of their insect antifeeding properties. We have isolated several types of limonoid antifeedants from some Meliaceae plants.^{1,2)} We have also recently reported the isolation of a C-19/C-29 bridged acyl acetal (azedarachin B), three azadirachtin-type compounds (meliacarpinins A, C and D),³⁾ and three ring C-seco limonoids (nimboldins C, D and E)⁴⁾ from *Melia toosendan*, which is native to China and a closely related plant to typical *M. azedarach*.

In our continuing investigation on the limonoid constituents, we isolated four new C-19/C-29 bridged acetals, 1-*O*-acetyltrichilin H (1) and neoazedarachins A (2), B (3) and D (4), as insect antifeedants together with two known compounds, 12 α -hydroxyamoorastatone (5)⁵⁾ and its 12-acetate, *iso*-chuanliansu (6),⁶⁾ which had earlier been isolated from *M. azedarach*. Their structures were elucidated by spectroscopic and chemical means. The present paper describes the isolation, structural elucidation and insect antifeedant activities by a conventional leaf disk method.

After a combination of droplet counter-current chromatography (DCCC) in an ascending mode, silica flash chromatography, preparative TLC and reversed-phase HPLC separations, a precipitate insoluble in 50% hexane/ether from the ethereal extract of the air-dried root bark gave six limonoids (1–6). Two known compounds 5 and 6 were isolated for the first time as an equilibrium mixture of *exo*- and *endo*-isomers at C-29 in ratios of about 7:3 and 4:1, respectively, only their *exo*-isomers having been previously reported.^{5,6)} Their structures were identified as 12 α -hydroxyamoorastatone (5) and its

12-acetate (6) by comparing their published ¹H-NMR data and further NMR studies containing nuclear Overhauser effect (NOE) measurements and ¹H-¹H and ¹³C-¹H COSY spectra.

Compound 1 was assigned the molecular formula C₃₈H₄₈O₁₅ according to the MH⁺ ion at *m/z* 745 from the SI-MS and ¹H-NMR data (Table). The presence of 9 methyl signals at δ 0.87, 1.14 and 1.24 (tertiary C-methyls), 1.20 and 1.21 (secondary C-methyls) and 1.90, 1.98, 2.05 and 2.11 (acetate methyls) was observed in the ¹H-NMR spectrum. It also showed the presence of a 14,15-epoxy [15-H: δ 3.72 (1H, s)] and a C-19/C-29 bridged acyl acetal structure [19-H₂: δ 4.34 and 4.45 (each 1H, d, *J*=13.6 Hz); and 29-H: δ 5.78 (1H, s)] together with a β -substituted furan moiety [δ 7.33, 7.12 and 6.09 (each 1H)]. Taking account the IR (3600–3300, 1740 and 1710 cm⁻¹) and CD ($\Delta\epsilon_{217}$ +13.7, $\pi \rightarrow \pi^*$ of furan; and $\Delta\epsilon_{309}$ –4.1, $n \rightarrow \pi^*$ of 11-keto) data, this information led to the assumption that 1 was a trichilin type of limonoid. A comparison of the ¹H-NMR data with those of known trichilins showed that 1 was almost superimposable on trichilin H (7), which had been isolated from *M. azedarach*,⁷⁾ except for the presence of one additional acetyl group. Compound 1 as 1-*O*-acetyltrichilin H was elucidated from couplings between the 1 β -, 2 β - and 3 β -H signals and the fact that the 9-H signal was shifted upfield to δ 4.17 in 1 from δ 4.64 in 7, due to the

Fig. 1. Structures of Trichilins, Azedarachins and Neoazedarachins.

release from the effect of the 1 α -hydroxyl group in the 1,3-diaxial relationship in **7**. The *S* configuration at C-29 was established from the chemical shifts of the 3 β - and 6 β -H signals at δ 5.70 and 2.04, which were largely influenced by the orientation of the 29-*O* function.⁸⁾ W-Shape long-range couplings between the 5-H and one (δ 4.45) of the 19-methylene signals and the 9-H and 30-H₃ signals, and NOE connectivities between the 18-H₃ signal and the 9, 21 and 22-H signals supported the stereochemistry of **1**.

Compound **2** was found by accurate mass measurement [HRFAB-MS *m/z*: 639.2799 (MNa⁺) (Δ mmu + 1.7)] to have the molecular formula C₃₃H₄₄O₁₁. The CD spectrum ($\Delta\epsilon_{295}$ –24.5, $\Delta\epsilon_{304}$ –23.1 and $\Delta\epsilon_{314}$ –14.2, $n \rightarrow \pi^*$ of keto groups) suggested the presence of plural carbonyl groups. The ¹H-(Table) and ¹³C-NMR spectra showed the presence of a C-19/C-29 bridged acyl acetal structure [19-H₂, δ 3.97 and 4.14 (each 1H, d, *J*=12.0 Hz); and 29-H, δ 5.77 (1H, s), δ 63.9 t (C-19) and δ 94.4 d (C-29)] together with three tertiary Me [δ 0.82 (28-H₃), 0.88 (18-H₃) and 1.21 (30-H₃)], one acetyl (δ 2.10) and a β -substituted furan moiety [δ 7.38, 7.30 and 6.42 (each 1H)]. The acyloxy side chain attached to C-29 was 2-methylbutanoyloxy in **2**, being different from 2-methylpropanoyloxy in **1**. The spectral data for **2** were closely related to those of azedarachin A (**8**), which had been isolated from *M. azedarach*,⁸⁾ having the same molecular formula. However, the ¹H- and ¹³C-NMR spectra of **2** showed the presence of an additional carbonyl group at δ 219.0 instead of the epoxide at δ 71.4 s and δ 59.5 d in **8** and a signal at δ 3.77 (1H, s) that was assigned to

the 15-H in **8** disappeared while a new signal was observed at δ 3.09 (br. s) in **2**. These signals strongly suggested the presence of a 15-keto group, which is consistent with the CD data.

The structure of **2** was finally elucidated from the transformation of **8** to **2** by treating with a catalytic amount of TsOH. This acid-catalyzed rearrangement of the D ring is known in limonoids.⁹⁾ The stereochemistry at C-14 was confirmed from the chemical shifts of 14 α -H at δ 3.09 and of 7 β -H at δ 4.24, the downfield shifts of both signals being attributable to the effect of 7-OH in a 1,3-diaxial relationship and to the anisotropic effect of the 15-keto group, respectively.

The structure of **3**, having the molecular formula C₃₂H₄₂O₁₁ assigned from the (MNa⁺) ion at *m/z* 625.2648 in the HRFAB-MS spectrum, was readily established by analogy with **2** since similar Cotton effects ($\Delta\epsilon_{294}$ –24.3, $\Delta\epsilon_{305}$ –22.7 and $\Delta\epsilon_{313}$ –16.2) were observed in the CD spectrum and since the proton shifts of rings A, B, C and D of both compounds corresponded to each other. The only difference was the acyl side chain at C-29, which was 2-methylpropanoyl in **3** and 2-methylbutanoyl in **2**. Compound **3** being the D-ring keto isomer of azedarachin B (**9**),³⁾ which had earlier been isolated from *M. toosendan*, was also confirmed from the formation of **3** by an acid treatment of **9**.

The ¹H-NMR spectrum of compound **4** (Table) having the molecular formula C₂₉H₃₈O₁₀ [(–) HRFAB-MS *m/z*: 545.2387 [M–H][–] (Δ mmu 0.0)], showed a three-proton singlet at δ 3.32 assignable to a methoxy group, together with three tertiary methyl signals. A compari-

Table ¹H-NMR Spectral Data for Compounds 1–4 (400 MHz, CDCl₃)

Position	1	2	3	4
1	5.45 (d, 4.4)*	4.77 (br. d, 4.5)	4.78 (m)	4.78 (br. d, 4.4)
2 α	—	1.96 (br. d, 16.0)	1.95 (dt, 16.4, 1.5)	1.85 (br. d, 16.1)
β	5.97 (t, 4.4)	2.82 (dt, 16.0, 4.2)	2.83 (dt, 16.4, 4.7)	2.85 (dt, 16.1, 4.4)
3	5.70 (d, 4.4)	5.35 (br. d, 3.8)	5.37 (dd, 4.7, 1.5)	4.90 (d, 4.0)
5	2.85 (dd, 13.9, 4.4)	2.79 (br. dd, 14.2, 3.0)	2.80 (br. dd, 14.3, 3.9)	2.57 (br. d, 14.3)
6 α	1.73 (br. d, 14.4)	1.78 (dt, 14.0, 3.9)	1.78 (dt, 13.8, 3.9)	1.71 (dt, 12.5, 3.3)
β	2.04 (m)	2.15 (dt, 2.0, 14.1)	2.13 (br. dt, 14.3, 2.0)	2.49 (dt, 2.2, 13.8)
7	3.70 (m)	4.24 (m)	4.24 (m)	4.18 (m)
9	4.17 (s)	3.44 (s)	3.44 (s)	3.43 (s)
12	5.46 (s)	4.42 (s)	4.42 (d, 2.0)	4.44 (s)
14	—	3.09 (br. s)	3.08 (br. s)	3.10 (br. s)
15	3.72 (s)	—	—	—
16 α	1.89 (dd, 13.9, 11.1)	2.61 (ddd, 18.0, 9.4, 1.9)	2.61 (ddd, 17.8, 9.6, 1.6)	2.59 (ddd, 17.3, 8.9, 1.7)
β	2.25 (dd, 13.9, 6.5)	2.70 (dd, 18.0, 8.2)	2.70 (dt, 17.8, 8.3)	2.70 (dd, 17.3, 8.4)
17	2.97 (dd, 11.1, 6.1)	3.51 (t, 9.0)	3.51 (t, 8.9)	3.51 (t, 8.6)
18	1.24 (s)	0.88 (s)	0.88 (s)	0.87 (s)
19a	4.34 (d, 13.6)	3.97 (br. d, 12.0)	3.97 (br. d, 12.5)	3.65 (br. d, 12.1)
b	4.45 (br. d, 13.6)	4.14 (d, 12.0)	4.14 (d, 12.5)	4.05 (d, 12.1)
21	7.12 (br. s)	7.30 (br. s)	7.30 (br. s)	7.30 (br. s)
22	6.09 (br. d, 2.2)	6.42 (m)	6.42 (br. d, 1.5)	6.41 (m)
23	7.33 (br. t, 1.5)	7.38 (br. t, 1.6)	7.38 (br. t, 1.7)	7.38 (t, 1.7)
28	0.87 (s)	0.82 (s)	0.82 (s)	0.86 (s)
29	5.78 (s)	5.77 (s)	5.76 (s)	4.19 (s)
30	1.14 (s)	1.21 (s)	1.21 (s)	1.21 (s)
Ac	1.90 (s), 1.98 (s) 2.05 (s), 2.11(s)	2.10 (s)	2.10 (s)	2.09 (s)
29-side chains				
2'	2.67 (sept, 7.0)	2.44 (ddq, 14.0, 7.0, 7.0)	2.62 (sept, 7.3)	—
2'-Me	1.20 (d, 7.0)	1.16 (d, 7.1)	1.19 (d, 7.3)	—
3'	1.21 (d, 7.0)	1.69 (tq, 7.0, 7.5)	1.20 (d, 7.3)	—
	—	1.50 (m)	—	—
3'-Me	—	0.92 (t, 7.0)	—	—
OMe	—	—	—	3.32 (s)

* Chemical shift (multiplicity, coupling constant in Hz).

son of the spectral properties of **4** with those of **2** and the CD Cotton effect ($\Delta\epsilon_{227} + 4.4$, $\Delta\epsilon_{295} - 19.7$, $\Delta\epsilon_{305} - 16.4$ and $\Delta\epsilon_{316} - 9.9$) due to the keto functions in **4** indicated that they were also closely related, including the C-19/C-29 bridged acetal and C-15 keto structures. However, the ^1H -NMR spectrum of **4** lacked the 2-methylbutanoyl signals and showed the presence of a methoxy signal, with an upfield shift of the 29-H signal to δ 4.19 in **4** from δ 5.77 in **2**. These data showed that compound **4** was a 29-*O*-methyl derivative of amoorastatone (**5**).

The *endo* configuration of the methoxy group was elucidated from the W-shape long-range coupling between the 29- and 5-H signals and the chemical shift change of the 3 β - and 6 β -H signals at δ 5.35 and δ 2.15 in **2** to δ 4.90 and δ 2.49 in **4**, respectively. Acetylation of **4** gave a 7,12-diacetate (**10**), in which the 14-H signal was shifted upfield to δ 2.81 from δ 3.10 in **4** to confirm its configuration to be α .

On the other hand, the α -configuration of the 12-hydroxyl group was unambiguously assigned from the chemical shifts of the furan protons and from the results of a CD study of 12-*p*-bromobenzoate **11**. The furan signals were observed at higher fields in the benzoate. Thus, the chemical shifts for compound **4**/its 12-benzoate were as follows: 21-H, 7.30/7.21; 22-H 6.41/6.20; and 23-H 7.38/7.28. The upfield shifts of the furan ring protons can be accounted for by the ring current of the benzoate aromatic ring which is located on the same side as the furan ring. On the other hand, the CD spectrum of **11** showed negatively split interaction bands between the benzoate and furan chromophores at 246 ($\Delta\epsilon - 3.5$, $\pi \rightarrow \pi^*$ transition of the benzoate) and 210.5 nm ($\Delta\epsilon + 6.1$, $\pi \rightarrow \pi^*$ transition of the furan) and negative carbonyl bands at 306 ($\Delta\epsilon - 3.9$) and 320 nm ($\Delta\epsilon - 2.7$) due to $n \rightarrow \pi^*$ transitions of the 11- and 15-keto groups (Fig. 2). This negatively split Cotton effect would be in accordance with the negative chirality between the benzoate and furan chromophores.¹⁰⁾

Finally, the structure of **4** was confirmed from *O*-methylation of 12-hydroxyamoorastatone (**5**) with a catalytic amount of TsOH in dried MeOH, which afforded **4** as the main product. Compound **4** is the first natural 29-*endo* derivative to be found in C-19/C-29 bridged acetal limonoids.

The antifeedant activity of the isolated compounds was tested against third-instar larvae of *Spodoptera litoralis* (Boisduval) by the conventional leaf disk method.¹¹⁾ The most potent were meliacarpinins, which were active at 50 ppm,¹²⁾ corresponding to a concentration of *ca.* 1 $\mu\text{g}/\text{cm}^2$. Azedarachin B (**9**) was the next most active at 200 ppm, followed by 12-hydroxyamoorastatone (**5**, 250 ppm), *iso*-chuanliansu (**6**, 300 ppm), and 1-*O*-acetylrichilin H (**1**, 400 ppm) and neoazedarachins (**2-4**, 400 ppm). These results show that isomerization of the D-ring epoxide to a 15-keto and acylation of the 12- and 29-OH groups reduced the activity, but that the side-chain change at C-29 did not influence their activity.

Fig. 2. CD and UV Spectra of **11**.

Experimental

Instrumental analyses. ^1H - and ^{13}C -NMR spectra were measured with a JEOL FX-400 spectrometer. IR and UV spectra were recorded with JASCO FT/IR 5300 and Shimadzu UV-210A spectrometers, respectively. Optical rotation was measured with a JASCO J-20A apparatus, and CD spectra with a JASCO J-720 spectropolarimeter. HPLC was performed on Waters μ Bondasphere 5 μ C18 100 Å in a 19 $\times$ 150 mm column and TLC on Kieselgel 60 (Merck).

Plant materials. The root bark was collected in December 1992 at Xiangtan in China.

Isolation of the limonoids. The air-dried root bark (1.5 kg) was defatted with hexane (20 l) and then extracted with ether (20 l) to give an extract of 12.8 g. The precipitate, which was insoluble in 50% hexane-ether, from the ethereal extract, was subjected to DCCC by using CH_2Cl_2 -MeOH- H_2O (5:5:3, v/v) in an ascending mode, and the resulting limonoid fraction was flash chromatographed on SiO_2 with a 50–100% ether/hexane solvents. HPLC purification of the limonoid fractions, which was eluted with ether, on a semiprep. reversed-phase column with 25–50% H_2O /MeOH as the solvents, gave **1** (0.8 mg), **2** (3.0 mg), **3** (0.9 mg), and meliacarpinins A (2.1 mg), C (5.2 mg) and D (6.2 mg). The more-polar DCCC fractions were subjected to prep. TLC with 50% benzene/acetone as the solvent, subsequent HPLC purification of the limonoid fraction in a similar manner to that just described giving **4** (16.5 mg), **5** (18.5 mg) and **6** (15.1 mg).

1-*O*-acetylrichilin H (1**).** An amorphous powder, $\text{C}_{38}\text{H}_{48}\text{O}_{15}$; $[\alpha]_{\text{D}} - 3.4^\circ$ (c 0.08, MeOH); UV λ_{max} (MeOH)

nm (ϵ): 210 (5000); IR $\nu_{\max}$ (KBr) cm^{-1} : 3600–3300, 1740, 1710, 1630 and 1620; CD: $\Delta\epsilon_{217} + 13.7$ ($\pi \rightarrow \pi^*$ of furan) and $\Delta\epsilon_{309} - 4.1$ ($n \rightarrow \pi^*$ of 11-keto); SI-MS m/z : 745 (MH^+).

Neoazedarachin A (2). An amorphous powder, $\text{C}_{33}\text{H}_{44}\text{O}_{11}$; $[\alpha]_{\text{D}}^{26}$ (c 0.2 MeOH); UV $\lambda_{\max}$ (MeOH) nm (ϵ): 215 (5100); IR $\nu_{\max}$ (KBr) cm^{-1} : 3600–3300, 1734, 1710 and 1637; CD: $\Delta\epsilon_{223} + 22.4$ ($\pi \rightarrow \pi^*$ of furan), and $\Delta\epsilon_{295} - 24.6$, $\Delta\epsilon_{304} - 23.1$ and $\Delta\epsilon_{314} - 14.2$ ($n \rightarrow \pi^*$ of ketones); SI-MS m/z : 617 (MH^+); (+) HRFAB-MS m/z (MNa^+): calcd. for $\text{C}_{33}\text{H}_{44}\text{O}_{11}\text{Na}$, 639.2782; found, 639.2799; NMR δ_{C} (CDCl_3): 11.7 q (3'-Me), 16.8 q (2'-Me), 19.0 q (C-28), 21.6 q (Ac), 21.7 q (C-18), 22.8 q (C-30), 23.2 t (C-6), 26.8 t (C-3'), 28.6 d (C-2'), 36.1 t (C-2), 39.5 s (C-10), 41.3 d (C-5), 41.3 s (C-8), 41.7 d (C-17), 43.5 s (C-4), 44.9 t (C-16), 45.7 s (C-13), 48.8 d (C-9), 60.9 d (C-14), 64.2 t (C-19), 69.7 d (C-7), 70.8 d (C-1), 73.9 d (C-3), 79.3 d (C-12), 94.2 d (C-29), 111.3 d (C-22), 124.2 s (C-20), 140.4 d (C-21), 143.4 d (C-23), 170.1 s (Ac), 175.6 s (C-1), 212.4 s (C-11) and 219.0 s (C-15).

Neoazedarachin B (3). An amorphous powder, $\text{C}_{32}\text{H}_{42}\text{O}_{11}$; $[\alpha]_{\text{D}}^{41}$ (c 0.09, MeOH); UV $\lambda_{\max}$ (MeOH) nm (ϵ): 219 (5300); IR $\nu_{\max}$ (KBr) cm^{-1} : 3600–3200, 1736, 1710 and 1637; CD: $\Delta\epsilon_{223} + 23.5$ ($\pi \rightarrow \pi^*$ of furan), and $\Delta\epsilon_{294} - 24.3$, $\Delta\epsilon_{305} - 22.7$ and $\Delta\epsilon_{313} - 16.2$ ($n \rightarrow \pi^*$ of ketones); SI-MS m/z : 603 (MH^+); (+) HRFAB-MS m/z (MNa^+): calcd. for $\text{C}_{32}\text{H}_{42}\text{O}_{11}$, 625.2625; found, 625.2648.

Neoazedarachin D (4). An amorphous powder, $\text{C}_{29}\text{H}_{38}\text{O}_{10}$; $[\alpha]_{\text{D}}^{13}$ (c 0.09, MeOH); UV $\lambda_{\max}$ (MeOH) nm (ϵ): 218 (3500); IR $\nu_{\max}$ (KBr) cm^{-1} : 3700–3200, 1734, 1710 and 1637; CD: $\Delta\epsilon_{227} + 4.4$ ($\pi \rightarrow \pi^*$ of furan), and $\Delta\epsilon_{295} - 19.7$, $\Delta\epsilon_{305} - 16.4$ and $\Delta\epsilon_{316} - 9.9$ ($n \rightarrow \pi^*$ of ketones); (–) HRFAB-MS m/z (M-H^-): calcd. for $\text{C}_{29}\text{H}_{37}\text{O}_{10}$, 545.2387; found, 545.2387; NMR δ_{C} (CDCl_3): 18.3 q (C-28), 21.2 q (C-18), 21.4 q (Ac), 22.4 q (C-30), 25.0 t (C-6), 25.8 d (C-5), 36.3 t (C-2), 40.4 s (C-10), 41.0 s (C-8), 41.2 d (C-17), 43.4 s (C-4), 44.7 t (C-16), 45.4 s (C-13), 48.7 d (C-9), 55.6 q (OMe), 57.7 t (C-19), 60.3 d (C-14), 69.6 d (C-7), 70.7 d (C-1), 76.3 d (C-3), 79.0 d (C-12), 102.9 d (C-29), 111.0 d (C-22), 121.4 s (C-20), 140.1 d (C-21), 143.0 d (C-23), 169.95 (Ac), 212.5 s (C-11) and 218.5 s (C-15).

Endo-isomer of 12-hydroxyamoorastatone (5). NMR δ_{H} ($\text{CDCl}_3 + \text{CD}_3\text{OD}$): 0.82 (3H, s, $4\alpha\text{-CH}_3$), 0.83 (3H, s, 13-CH_3), 1.10 (3H, s, 8-CH_3), 1.69 (1H, m, $6\alpha\text{-H}$), 1.78 (1H, dt, $J=16.1$ and 1.7 Hz, $2\alpha\text{-H}$), 1.99 (1H, m, $6\beta\text{-H}$), 2.03 (3H, s, COOCH_3), 2.49 (1H, m, $16\alpha\text{-H}$), 2.58 (1H, dd, $J=15.3$ and 2.0 Hz, 5-H), 2.63 (1H, m, $16\beta\text{-H}$), 2.71 (1H, dt, $J=16.0$ and 4.8 Hz, $2\beta\text{-H}$), 2.78 (1H, dt, $J=16.1$ and 1.7 Hz, $2\alpha\text{-H}$), 3.28 (1H, br s, 14-H), 3.38 (1H, t, $J=8.5$ Hz, 17-H), 3.53 (1H, s, 9-H), 3.77 (1H, d, $J=11.7$ Hz, 19-Ha), 4.18 (1H, s, 12-H), 4.31 (1H, d, $J=11.7$ Hz, 19-Hb), 4.52 (1H, br. d, $J=4.8$ Hz, 1-H), 4.62 (1H, s, 29-H), 4.71 (1H, dt, $J=16.0$ and 4.8 Hz), 6.27 (1H, br. s, 22-H), 7.24 (1H, br. s, 21-H) and 7.31 (1H, br. s, 23-H).

Endo-isomer of iso-chuanliansu (6). NMR δ_{H} ($\text{CDCl}_3 + \text{CD}_3\text{OD}$): 0.89 (3H, s, $4\alpha\text{-CH}_3$), 1.02 (3H, s, 8-CH_3), 1.20 (3H, s, 13-CH_3), 1.77 (1H, m, $2\alpha\text{-H}$), 1.77 (1H, m, $6\alpha\text{-H}$), 2.09, 2.12 (each 3H, s, COOCH_3), 2.50 (1H, dt, $J=2.5$ and 13.4 Hz, $6\beta\text{-H}$), 2.60 (2H, d, $J=9.4$ Hz, 16-H_2), 2.60 (1H, m, 5-H), 2.78 (1H, dt, $J=16.4$ and 4.5 Hz, $2\beta\text{-H}$), 3.32 (1H, s, 14-H), 3.34 (t, $J=9.0$ Hz, 17-H), 3.77 (1H, s, 9-H), 3.95 (1H, m, 7-H), 3.98 (1H, d, $J=12.0$ Hz, 19-Ha), 4.12 (1H, br. d, $J=4.0$ Hz, 1-H), 4.42 (1H, d, $J=12.0$ Hz, 19-Hb), 4.69 (1H, s, 29-H), 4.84 (1H, br. d, $J=4.0$ Hz, 3-H), 5.06 (1H, s, 12-H), 6.29 (1H, m, 22-H), 7.32 (1H, br. s, 21-H) and 7.40 (1H, br. t, $J=1.5$ Hz, 23-H).

Treatment of azedarachin A (8) with *p*-toluenesulfonic acid. Azedarachin A (8, 0.7 mg) was treated with a catalytic amount of TsOH in CH_2Cl_2 (1 ml) at r.t. for 3 h. The product was purified by semiprep. HPLC with 40% $\text{H}_2\text{O}/\text{MeOH}$ as the solvent to give 2 (0.4 mg).

Treatment of azedarachin B (9) with *p*-toluenesulfonic acid. Azedarachin B (9, 1 mg) was treated with a catalytic amount of TsOH in CH_2Cl_2 (1 ml) at r.t. for 1 h to give 3 (0.3 mg).

Acetylation of neoazedarachin D (4). Compound 4 (2 mg) was acetylated with Ac_2O (0.1 ml) in Py (0.5 ml) at r.t. for 4 d. The reaction mixture was washed with H_2O , and the product was purified by semiprep. HPLC with 30% $\text{H}_2\text{O}/\text{MeOH}$ as the solvent to give 10 (0.3 mg), $\text{C}_{33}\text{H}_{42}\text{O}_{12}$; FAB-MS: m/z 613 (MH^+); selected NMR δ_{H} (CDCl_3): 0.79 (3H, s, $4\alpha\text{-CH}_3$), 1.03 (3H, s, 13-CH_3), 1.24 (3H, s, 8-CH_3), 2.05, 2.10, 2.12 (each 3H, s, COCH_3), 2.81 (1H, s, 14-H), 3.29 (1H, t, $J=9.0$ Hz, 17-H), 3.31 (3H, s, OCH_3), 3.73 (1H, s, 9-H), 3.95, 4.12 (each 1H, d, $J=11.3$ Hz, 19-H_2), 4.17 (1H, s, 29-H), 4.25 (1H, d, $J=4.4$ Hz, 1-H), 4.89 (1H, d, $J=4.4$ Hz, 2-H), 5.24 (1H, s, 12-H), 5.26 (1H, m, 7-H), 6.36, 7.30 and 7.39 (each 1H, furan-H).

Benzoylation of neoazedarachin D (4). Compound 4 (4.0 mg) was treated with *p*-bromobenzoyl chloride (10 mg) in Py (1 ml) at 50°C for 4 d. After washing with H_2O , the reaction products were subjected to prep. TLC, using 50% $\text{Me}_2\text{CO}/\text{C}_6\text{H}_6$ as the solvent, and the limonoid fraction was purified by semiprep. HPLC with 20% $\text{H}_2\text{O}/\text{MeOH}$ as the solvent to give 12-benzoate 11 (1.0 mg), $\text{C}_{36}\text{H}_{41}\text{BrO}_{11}$; FAB-MS m/z : 713 and 715 (MH^+); UV $\lambda_{\max}$ (MeOH) nm (ϵ) 207 (9000) and 245 (8700); CD: $\Delta\epsilon_{210.5} + 6.1$ ($\pi \rightarrow \pi^*$ of furan), $\Delta\epsilon_{246}$ ($\pi \rightarrow \pi^*$ of benzoate), and $\Delta\epsilon_{306} - 3.9$ and $\Delta\epsilon_{320} - 2.7$ ($n \rightarrow \pi^*$ of ketones); selected NMR δ_{H} (CDCl_3): 0.84 (3H, s, $4\alpha\text{-CH}_3$), 1.12 (3H, s, 13-CH_3), 1.27 (3H, s, 8-CH_3), 3.23 (1H, 14-H), 3.31 (3H, s, OCH_3), 3.40 (1H, br. t, $J=9.6$ Hz, 17-H), 3.61 (1H, s, 9-H), 3.84, 4.09 (each 1H, d, $J=12.0$ Hz, 19-H_2), 4.15 (1H, s, 29-H), 4.39 (1H, br. d, $J=4.1$ Hz, 1-H), 4.86 (1H, br. d, $J=4.3$ Hz, 3-H), 5.61 (1H, s, 12-H), 6.20, 7.21, 7.28 (each 1H, furan-H), 7.55 (2H, dt, $J=8.6$ and 1.9 Hz, $2'$ and $6'\text{-H}$ of benzoate) and 7.78 (2H, dt, $J=8.6$ and 1.9 Hz, $3'$ and $5'\text{-H}$ of benzoate).

Methylation of 12-hydroxyamoorastatone (5). To a solution of compound **5** (4 mg) in dried MeOH (1 ml) was added a catalytic amount of TsOH. The reaction mixture was stirred at r.t. for 18 h, poured into ice-cooled water, and extracted with ether. The ethereal layer was successively washed with a 5% Na₂CO₃ solution and water, and concentrated. The residue was purified by semiprep. HPLC with 30–35% H₂O/MeOH as the solvent to give **4** (1.0 mg).

Bioassay of the limonoid antifeedants. The antifeedant potential of the isolated compounds was tested by the conventional leaf disk method¹²⁾ against third-instar larvae of *S. littoralis* (Boisduval). Five disks of Chinese cabbage (*Brassica campestris* L. var *chinensis*) treated with the sample solution in Me₂CO were arranged with another 5 control disks immersed in Me₂CO alone in a Petri dish. Ten larvae were placed in the center, and the score for the treated and untreated leaves eaten by the larvae in 2–24 h was evaluated at appropriate intervals. From these choice tests at 50, 100, 200, 250, 300 and 400 ppm, the minimum inhibitory concentration of each limonoid was determined.

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