The Structures of Azedarachins, Limonoid Antifeedants from Chinese *Melia azedarach* Linn

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Three azedarachins, insect antifeedant limonoids, have been isolated from the root bark of Chinese *Melia azedarach* Linn (Meliaceae), along with six trichilins. Their structures show a 14,15-epoxide and a 19/29 acyl acetal bridge system, as elucidated by spectroscopic studies. They are antifeedants active against the larvae of *Spodoptera exigua* Hübner (Boisduval).

Meliaceae plants are a rich source of limonoid and the neem tree *Melia azadirachta indica* Juss and related Meliaceae tree *Melia azedarach* Linn are attracting considerable interest, particularly because of their insect antifeedant properties. *M. azedarach* is a native of Persia, India, and China, but has become naturalized on a number of continents including Africa, Australia, and Americas. Thus, the constituents of the tree have been studied in Nigeria, Australia, China, the U. S. A. and Japan, and many different types of limonoids have been isolated; i.e., degraded limonoids,[1] azadirachtin,[2] and related compounds,[3] trichilin-type 19/29 bridged acyl acetals,[4] toosendanin[5] (chuanliansu[6]) and related compound,[6] and salannin-type C-seco limonoids,[7] etc.

Recentry, we isolated new meliacarpinins as insect antifeedant from Okinawan[8] and Chinese[9] *M. azedarach* L. In the study of limonoid antifeedants from the Chinese plant, we have isolated three new sendanin-type[10] limonoids, **1**—**3**, along with six trichilins, **4**—**9**, including three new compounds.[11] These first natural sendanin-type 19/29 bridged acyl acetals, designated as azedarachins, showed antifeedant activities at 200—400 ppm, corresponding to the concentration of 4—8 $\mu g\,cm^{-2}$, by a conventional leaf disk method[12] against the larvae of the voracious pest insect *Spodoptera exigua* Hübner (Boisduval).

Results and Discussion

The ether extract of the root bark contained a variety of limonoids which were detected by the characteristic color with Ehrlich's reagent on TLC. The antifeeding limonoids from *M. azedarach* were also very sensitive to traces of acid and gradually decomposed on a silica column.[13] It was, therefore, necessary to use flash chromatography and HPLC separation techniques, and the isolation of the various congeners, **1**—**9**, was a tedious process requiring careful use of HPLC.

A powder, insoluble in 50% hexane/ether, from the ether extract of the root bark (375 g) was flash-chromatographed with 1% $MeOH/CH_2Cl_2$ and each of the limonoid fractions was rechromatographed on a flash column with 20% hexane/ether solvent system. Finally, very careful combined use of normal- and reversed-phase HPLC gave azedarachins: A (**1**; 1 mg) (Chart 1), 12-*O*-acetylazedarachin A (**2**; 0.7 mg) and 12-*O*-acetylazedarachin B (**3**; 2.5 mg) (Chart 2), and six trichilins, **4**—**9** (see Experimental, Chart 3).

Extensive ^1H and ^{13}C NMR studies of azedarachin A (**1**), $C_{33}H_{44}O_{11}$, SIMS *m/z* 639 $(M+Na)^+$ and 617 $(M+1)^+$, including COSY, DEPT spectra, and NOE experiments, taking account the circular dichroism (CD) data ($\Delta\varepsilon$ −26; n–π* of 11-oxo group), allowed us to assign all of the peaks in the complex spectra as well as to derive structure **1**. Some pertinent points related to the structural study for **1** are as follows. ^{13}C and ^1H (at 27 and 45 °C) NMR data indicated that **1** contained 6 CH_3, 5 CH_2, 13 CH, 9 carbons (1 ketone and 2 acyloxy) not bonded to hydrogen and 3 protons due to OH groups. The ^1H NMR (Table 1) revealed the presence of the typical 2-methylbutanoyl and acetyl substituents and 3-furyl moiety. The ^1H and ^{13}C (Table 2) NMR spectra indicated the presence of the 14,15-epoxide [δ=3.77 (s, 15α-H), 59.4d (C-15), and 71.4s (C-14)]

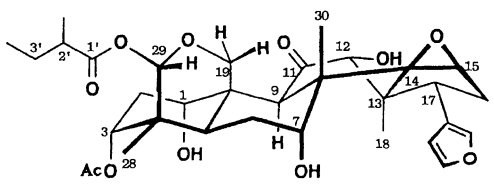
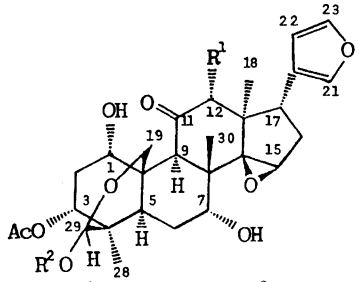


Chart 1. Azedarachin A (**1**).



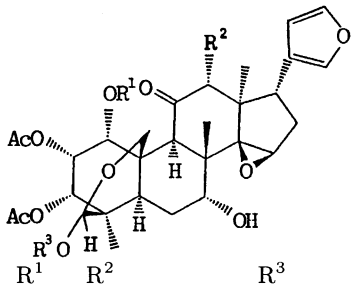
	R^1	R^2
1:	OH	$COCH(CH_3)CH_2CH_3$
2:	OAc	$COCH(CH_3)CH_2CH_3$
3:	OAc	$COCH(CH_3)_2$
10:	OAc	Ac

Chart 2.

Table 1. ^{1}H NMR Data for Azedarachins, **1**, **2**, and **3**, and Sendanin (**10**)[19]

	1	**2**	**3**	**10**
H	δ Mult (J/Hz)	δ Mult (J/Hz)	δ Mult (J/Hz)	δ Mult (J/Hz)
1	4.47*m*	4.27*m*	4.27*m*	4.28*m*
2α	1.89*br d* (15.0)	1.90*br d* (16.4)	1.89*br d* (16.4)	1.90*br d* (16.0)
2β	2.86*dt* (16.6, 4.9)	2.82*dt* (16.6, 4.7)	2.82*dt* (16.4, 4.6)	2.82*dt* (16.0, 4.7)
3	5.33*br d* (4.0)	5.30*d* (4.0)	5.31*br d* (4.4)	5.29*br d* (4.2)
5	2.72*dd* (14.0, 4.5)	2.71*dd* (14.3, 3.6)	2.72*dd* (13.9, 4.0)	2.73*dd* (14.0, 4.2)
6α	1.73*dt* (14.3, 3.7)	1.73*dt* (14.7, 3.7)	1.73*dt* (14.3, 3.7)	1.72*dt* (14.5, 3.6)
6β	2.02*dt* (2.0, 14.0)	2.04*dt* (2.1, 14.7)	2.04*dt* (2.1, 14.3)	2.02*dt* (2.1, 14.2)
7	3.66*m*	3.67*m*	3.67*m*	3.68*m*
9	4.52*s*	4.61*s*	4.61*s*	4.61*s*
12β	4.11*s*	5.28*s*	5.28*s*	5.28*s*
15	3.77*s*	3.75*s*	3.75*s*	3.75*s*
16α	2.35*dd* (13.4, 6.4)	2.25*dd* (13.7, 6.5)	2.25*dd* (13.2, 6.3)	2.24*dd* (13.3, 6.5)
16β	1.91*dd* (13.0, 11.0)	1.91*dd* (13.7, 11.6)	1.92*dd* (13.2, 11.1)	1.92*dd* (13.3, 11.3)
17	3.03*dd* (11.4, 6.2)	2.98*dd* (11.4, 6.0)	2.98*dd* (11.1, 6.1)	2.98*dd* (11.3, 6.5)
18(Me)	1.17*s*	1.32*s*	1.32*s*	1.32*s*
19a	4.26*d* (12.3)	4.27*d* (13.0)	4.28*d* (12.4)	4.30*d* (13.2)
19b	4.32*d* (12.3)	4.32*d* (13.0)	4.34*d* (12.4)	4.34*d* (13.2)
21	7.22*br d* (1.5)	7.13*br d* (1.4)	7.13*br d* (1.4)	7.12*br d*(1.5)
22	6.53*m*	6.15*m*	6.15*m*	6.14*m*
23	7.32*t* (1.5)	7.33*t* (1.5)	7.33*t* (1.6)	7.34*t* (1.5)
28(Me)	0.82*s*	0.84*s*	0.83*s*	0.82*s*
29	5.80*s*	5.81*s*	5.80*s*	5.79*s*
30(Me)	1.14*s*	1.17*s*	1.17*s*	1.13*s*
2′	2.45*sext* (7.0)	2.44*sext* (7.1)	2.61*sext* (7.0)	—
2′-Me	1.17*d* (7.0)	1.17*d* (7.4)	1.19*d* (7.0)	—
3′	1.71*hept* (7.0)	1.70*hept* (7.4)	1.20*d* (7.0)	—
3′	1.53*hept* (7.4)	1.51*hept* (7.4)	—	—
3′-Me	0.93*t* (7.6)	0.93*t* (7.6)	—	—
Ac	—	1.98*s*	1.98*s*	1.98*s*
	2.11*s*	2.11*s*	2.11*s*	2.11*s*
	—	—	—	2.12*s*
1-OH	3.07*d* (1.0)	2.32*d* (8.0)	2.32*d* (7.7)	2.32*d* (7.3)

Measured in $CDCl_3$ at 400 MHz.

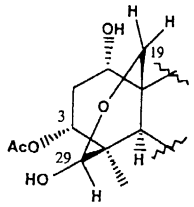


	R^1	R^2	R^3
4:	H	OH	$COCH(CH_3)CH_2CH_3$
5:	H	OAc	$COCH(CH_3)_2$
6:	H	OAc	$COCH(CH_3)CH_2CH_3$
7:	Ac	OAc	$COCH(CH_3)CH_2CH_3$
8:	H	H	$COCH(CH_3)CH_2CH_3$
9:	H	H	$COCH(CH_3)_2$

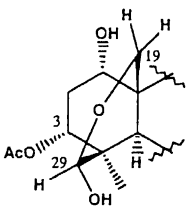
Chart 3.

and the 19/29 bridged acyl acetal ester system [δ=4.27 and 4.32 (each d, J=13.0 Hz, 19-H_2), 5.80 (s, 29-H), and 65.0t (C-19) and 94.6d (C-29)] like sendanin (**10**)[10] and trichilins (Chart 3).[14,15] Irradiation of the 8- and 13-Me peaks induced 9 and 6% NOEs on one (δ=4.32) of the 19-H_2 and the 7-H signals and 25, 8, and 5% NOEs on the 9-, 21-, and 22-H signals, respectively. Another peak of the 19-H_2 at δ=4.26 showed a W-type long range coupling with the 5-H, and the 1- and 9-H signals also showed long range couplings with 3-H and 8-Me signals, respectively. These data strongly suggested that **1** was 2-deacetoxytrichilin B. The substitution pattern around the A-ring, namely, that **1** has a free 1-OH and 3-acetoxyl groups, the same as trichilin B (**4**)[14] isolated together from the root bark, was shown by the fact that the 9-H signal in **1** was at δ=4.52, due to the effect of the 1-hydroxyl in a 1,3-diaxial relationship; δ=4.56 in **4**; in the 1-*O*-acetylated compounds, it was observed at δ=4.0—4.2.[15,16]

On the other hand, the fact that the 12-OH group in **1** is α was deduced from the chemical shift (δ=3.02) of the 17β-H, which was shifted down field to δ=3.39 in trichilin A (12β-OH).[14] Finally, the *S*-configuration at C-29 was assigned from the chemical shift of 3-H. As shown in Fig. 1, no apparent differences were observed on 19- and 29-H in *exo*- and *endo*-19/29 bridged hemi-



exo-



endo-

	exo-				*endo-*			
	3-H	29-H	19-H_2		3-H	29-H	19-H_2	
29-OH[19]	5.37 br d	4.79 s	4.33 d	4.25 d	4.90 br d	4.89 s	4.50 d	4.20 d
(Toosendanin)	(3.7)		(12.5)	(12.5)	(3.7)		(12.5)	(12.5)
29-OBz[5]	5.49	6.05 s	4.42 s		5.07	6.08 s	4.46 d	4.38 d
							(12)	(12)
29-OAc	5.29 br d	5.79 s	4.34 d	4.30 d				
(Sendanin)	(4.2)		(12.5)	(12.5)				
Azedarachins	5.3—5.35 br d	5.80—5.81 s	4.32—4.34 d	4.26—4.28 d				
	(4.0—4.4)		(12—13)	(12—13)				
Trichilins	5.5—5.6 br d	5.7—5.8 s	4.3—4.55 s	4.25—4.4 d				
(2α-OAc)	(4.2—5.0)		(12—13.5)	(12—13.5)				

Fig. 1. Configuration at C-29 and the chemical shifts of 3-, 19-, and 29-H signals.

Table 2. ^{13}C NMR Data of Azedarachins, **1** and **3**, and Sendanin (**10**)[19]

C	**1**	**3**	**10**	C	**1**	**3**	**10**
1	70.6*d*	70.1*d*	70.4*d*	20	121.1*s*	122.5*s*	122.8*s*
2	33.4*t*	33.6*t*	33.9*t*	21	142.6*d*	142.4*d*	142.8*d*
3	74.0*d*	73.6*d*	74.0*d*	22	113.1*d*	111.9*d*	112.2*d*
4	39.8*s*	39.5*s*	39.5*s*	23	141.0*d*	140.7*d*	141.0*d*
5	41.4*d*	34.1*d*	28.3*d*	28	19.5*q*	19.4*q*	19.6*q*
6	25.7*t*	25.8*t*	26.0*t*	29	94.6*d*	94.3*d*	94.8*d*
7	70.4*d*	70.5*d*	70.7*d*	30	14.7*q*	15.8*q*	16.1*q*
8	41.9*s*	41.5*s*	41.8*s*	1′	175.6*s*	175.7*s*	
9	48.1*d*	48.4*d*	48.7*d*	2′	28.3*d*	28.0*d*	
10	42.5*s*	42.5*s*	42.8*s*	2′-Me	16.8*q*	18.6*q*	
11	207.1*s*	206.6*s*	206.9*s*	3′	26.9*t*	18.9*q*	
12	79.2*d*	78.6*d*	78.9*d*	3′-Me	11.7*q*		
13	46.4*s*	45.9*s*	46.2*s*	Ac	21.8*q*	20.7*q*	21.0*q*
14	71.4*s*	72.0*s*	72.3*s*			21.5*q*	21.4*q*
15	59.5*d*	58.5*d*	58.8*d*				21.8*q*
16	35.7*t*	35.0*t*	35.4*t*		169.7*s*	169.8*s*	170.1*s*
17	38.9*d*	38.3*d*	38.7*d*			170.4*s*	170.2*s*
18	23.1*q*	22.3*q*	22.7*q*				170.7*s*
19	65.0*t*	64.6*t*	65.0*t*				

Measured in $CDCl_3$ at 100 MHz.

acetals and their acyl acetals, but, in **1**, where the *exo*-configuration of the 2-methylbutanoyl group has been established, the 3-H signal appeared at the low position of δ=5.33, as well as sendanin (**10**) and all of trichilins, compared to δ=4.90 in the *endo*-isomer of toosendanin. This assignment was also supported by an NOE observation between the 28-Me and 29-H signals.

The structure of the second limonoid **2**, $C_{35}H_{46}O_{12}$, SIMS *m/z* 681 $(M+Na)^+$ and 651 $(M+1)^+$, possessing one additional acetyl group along with a 3-furyl ring and a 2-methylbutanoyl group, was readily suggested to be 12-*O*-acetylazedarachin A (**2**) from the ^{1}H NMR spectrum, in which only one signal due to 12-H showed a large shift to δ=5.28 form δ=4.11 in **1**. We failed in the acetylation of the 12-OH group in **1**, but the fact that the 12-OAc in **2** is also α was deduced from an NOE connectivity (25% NOE) in the 17β-H and 12β-H signals. Interestingly, the 1-H signal was shifted upfield to δ=4.27 from δ=4.47 in **1**. This strongly suggested that the conformation around the ring C in **2** differed from that in **1**. This change could be attributed to the presence of a five-membered hydrogen bonding between the 11-oxo and 12-hydroxyl groups in **1**.

The ^{1}H NMR spectrum of the third limonoid **3**, $C_{34}H_{44}O_{12}$, SIMS *m/z* 667 $(M+Na)^+$ and 645 $(M+1)^+$, named 12-*O*-acetylazedarachin B, was almost superimposable on that of 12-*O*-acetylazedarachin A (**2**), except that the ester moiety at C-29 was 2-methylpropanoyl. The chemical shifts of some carbons due to the ring C, including 8- and 13-Me, were observed to be different from those of **1**. This is also suggestive of the comformation change around the ring C, in a similar manner to that above. It is particularly of interest that the signal due to C-5 seems to shift depending upon the change of the C-28 moiety.

The compounds, **1**, **2** and **3**, showed antifeedant activity against the larvae of Japanese pest insect *Spodoptera exigua* Hübner (Boisduval). The most potent is the compound with a 12-OH function (azedarachin A) which is active at 200 ppm, corresponding to the concentration of ca. 4 $\mu g\,cm^{-2}$, by the conventional leaf disk method.[12] The activity is less than those of azadirachtin and related limonoids[17] but comparable to that of trichilin B belonging to the second class limonoids. The 12-acetoxy compounds, **2** and **3**, were thus active at 400 ppm. These activities were almost independent of the substitution pattern in ring A and the 28-ester moiety.

Experimental

^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ on a JEOL FX-400 spectrometer. UV spectra were recorded in MeOH on a Shimadzu UV-210A spectrophotometer. Optical rotation and circular dichroism (CD) spectra were measured using a JASCO J-20A spectropolarimeter. HPLC was performed on Waters μPorasil and μBondasphere columns by using 0.7—2.0% MeOH–CH_2Cl_2 and 20—40% H_2O–MeOH as solvents.

Plant Material. The root bark was collected in October 1992 at Guangzhou, China.

Extraction and Isolation. The dried root bark (375 g) was extracted with ether to yield 3.1 g of an extract. This was dissolved in 13 ml of ether and then the same volume of hexane was added to give 975 mg of a precipitate. The resulting mixture containing limonoids was flash-chromatographed on SiO_2 with CH_2Cl_2 containing 1—10% MeOH, and the limonoid fractions eluted with 1—1.5% MeOH–CH_2Cl_2 were rechromatographed on a flash column with a hexane–ether solvent system. Each resulting limonoid fraction was separated through HPLC using normal and reversed columns to give the following limonoids: azedarachins, **1** (1 mg), **2** (0.7 mg) and **3** (2.5 mg) and trichilins, **4** (2 mg), **5** (9.5 mg), **6** (10 mg), **7** (1 mg), **8** (16 mg) and **9** (2.5 mg).

Azedarachin A (1). An amorphous powder; $[\alpha]_D^{22}$ $-10°$ (c 0.05, MeOH); UV (MeOH) 213 nm (ε 4300); CD (MeOH) 223 ($\Delta\varepsilon$ +6, π–π^* of furan +n–π^* of ester carbonyls), 310 nm ($\Delta\varepsilon$ −26, n–π^* of 11-keto); SIMS Found: m/z 639 $(M+Na)^+$, 617 $(M+1)^+$. Calcd for $C_{33}H_{44}O_{11}$: M, 616.

12-*O*-Acetylazedarachin A (2). An amorphous powder; $[\alpha]_D^{22}$ +7.5° (c 0.08, MeOH); UV (MeOH) 212 nm (ε 6300); CD (MeOH) 218 ($\Delta\varepsilon$ +17, π–π^* of furan +n–π^* of ester carbonyls), 309 nm ($\Delta\varepsilon$ −14, n–π^* of 11-keto group); SIMS Found: m/z 681 $(M+Na)^+$, 659 $(M+1)^+$. Calcd for $C_{35}H_{46}O_{12}$: M, 658.

12-*O*-Acetylazedarachin B (3). An amorphous powder; $[\alpha]_D^{22}$ $-55°$ (c 0.13, MeOH); UV (MeOH) 213 nm (ε 3000); CD (MeOH) 217 ($\Delta\varepsilon$ +25, π–π^* of furan +n–π^* of ester carbonyls), 308 nm ($\Delta\varepsilon$ −10, n–π^* of 11-keto); SIMS Found: m/z 667 $(M+Na)^+$, 645 $(M+1)^+$. Calcd for $C_{34}H_{44}O_{12}$: M, 644.

Trichilin B (4). $C_{35}H_{46}O_{13}$; $[\alpha]_D^{22}$ $-10°$ (c 0.08); UV 212 nm (ε 3100); CD 217 ($\Delta\varepsilon$ +7), 310 nm ($\Delta\varepsilon$ −12); ^{1}H NMR δ=0.83 (3H, s), 0.96 (3H, t, J=7.5 Hz), 1.14 (3H, s), 1.15 (3H, s), 1.19 (3H, d, J=7.0 Hz), 1.56 (1H, m), 1.71 (1H, m), 1.74 (1H, dt, J=13.8, 3.6 Hz), 1.90 (1H, dd, J=13.3, 11.3 Hz), 2.00 (1H, dt, J=2.2, 13.8 Hz), 2.02 (3H, s), 2.12 (3H, s), 2.35 (1H, dd, J=13.3, 6.5 Hz), 2.40 (1H, d, J=3.7 Hz), 2.51 (1H, sext, J=7.0 Hz), 2.85 (1H, dd, J=14.0, 3.6 Hz), 3.02 (1H, dd, J=11.3, 6.0 Hz), 3.66 (1H, m), 3.78 (1H, s), 4.11 (1H, s), 4.31 (1H, d, J=12.8 Hz), 4.36 (1H, d, J=12.8 Hz), 4.56 (1H, s), 5.53 (1H, d, J=4.4 Hz), 5.91 (1H, t, J=4.4 Hz), 6.53 (1H, m), 7.22 (1H, br s), 7.33 (1H, t, J=1.6 Hz); ^{13}C NMR δ=11.3q, 16.3q, 18.6q, 20.6q, 20.8q, 22.7q, 25.3q, 26.4t, 27.7d, 33.1t, 38.6d, 40.7s, 41.2d, 42.3s, 42.4s, 46.2d, 47.3s, 59.2d, 64.1t, 68.6d, 70.0s, 71.7d, 72.8d, 73.0d, 78.9d, 93.5d, 112.8d, 123.5s, 140.8d, 142.3d, 169.0s, 170.1s, 175.3s, 213.2s; CIMS m/z 675 $(M+1)^+$.

Trichilin H (5). $C_{36}H_{46}O_{14}$; $[\alpha]_D^{22}$ $-20°$ (c 0.12); UV 213 nm (ε 5500); CD 220 ($\Delta\varepsilon$ +45); 308 nm ($\Delta\varepsilon$ −21); ^{1}H NMR δ=0.84 (3H, s), 1.17 (3H, s), 1.21 (3H, d, J=7.0 Hz), 1.22 (3H, d, J=7.0 Hz), 1. 35 (3H, s), 1.74 (1H, dt, J=14.3, 3.6 Hz), 1.90 (1H, dd, J=13.3, 11.4 Hz), 1.98 (3H, s), 2.02 (3H, s), 2.03 (1H, m), 2.13 (3H, s), 2.24 (1H, dd, J=13.3, 6.1 Hz), 2.51 (1H, d, J=4.0 Hz), 2.67 (1H, hept, J=7.0 Hz), 2.81 (1H, dd, J=3.7, 13.3 Hz), 2.99 (1H, dd, J=11.3, 6.1 Hz), 3.68 (1H, s), 4.31 (1H, d, J=13.6 Hz), 4.38 (1H, d, J=13.6 Hz), 4.42 (1H, t, J=4.2 Hz), 4.64 (1H, s), 5.40 (1H, s), 5.53 (1H, d, J=4.5 Hz), 5.75 (1H, s), 5.90 (1H, t, J=4.6 Hz), 6.12 (1H, br s), 7.13 (1H, s), 7.34 (1H, br s); ^{13}C NMR δ=15.5q, 18.7q, 18.7q, 18.8q, 20.7q, 20.7q, 20.9q, 22.4q, 25.6t, 27.7d, 33.8t, 34.1d, 38.1d, 40.7s, 42.3s, 42.5s, 45.6s, 48.0d, 58.8d, 64.1t, 68.5d, 70.2d, 71.6d, 71.9s, 73.0d, 77.9d, 93.5d, 111.9d, 122.5s, 140.7d, 142.4d, 168.9s, 170.0s, 170.2s, 175.7s, 206.3s; CIMS m/z 703 $(M+1)^+$.

12-*O*-Acetyltrichilin B (6). $C_{37}H_{48}O_{14}$; $[\alpha]_D^{22}$ $-2.5°$ (c 0.16); UV 211 nm (ε 4000); CD 217 ($\Delta\varepsilon$ +29), 308 nm ($\Delta\varepsilon$ −21); ^{1}H NMR δ=0.85 (3H, s), 0.93 (3H, t, J=7.1 Hz), 1.17 (3H, s), 1.18 (3H, d, J=7.0 Hz), 1.35 (3H, s), 1.55 (1H, m), 1.72 (1H, m), 1.73 (1H, dt, J=14.3, 3.8 Hz), 1.90 (1H, dd, J=13.3, 11.2 Hz). 1.98 (3H, s), 2.02 (3H, s), 2.13 (3H, s), 2.24 (1H, dd, J=13.3, 6.4 Hz), 2.50 (1H, d, J=3.9 Hz), 2.51 (1H, sext, J=6.9 Hz), 2.81 (1H, dd, J=13.9, 3.5 Hz), 2.99 (1H, dd, J=10.7, 5.7 Hz), 3.68 (1H, m), 3.76 (1H, s), 4.30 (1H, d, J=12.0 Hz), 4.37 (1H, d, J=12.0 Hz), 4.42 (1H, dd, J=4.4, 4.0 Hz), 4.63 (1H, s), 5.42 (1H, s), 5.52 (1H, d, J=4.7 Hz), 5.77 (1H, s), 5.90 (1H, t, J=4.6 Hz), 6.13 (1H, br d, J=1.6 Hz), 7.13 (1H, m), 7.34 (1H, t, J=1.5 Hz); SIMS m/z 717 $(M+1)^+$.

1,12-Di-*O*-acetyltrichilin B (7). $C_{39}H_{50}O_{15}$; $[\alpha]_D^{22}$ +0.8° (c 0.08); UV 211 nm (ε 5200); CD 207 ($\Delta\varepsilon$ +34), 311 nm ($\Delta\varepsilon$ −9); ^{1}H NMR δ=0.88 (3H, s), 0.93 (3H, t, J=7.4 Hz), 1.14 (3H, s), 1.18 (3H, d, J=14.0 Hz), 1.24 (3H, s), 1.53 (1H, m), 1.71 (1H, hept, J=7.0 Hz), 1.73 (1H, br d, J=14.0 Hz), 1.89 (1H, dd, J=13.1, 11.0 Hz), 1.90 (3H, s), 1.98 (3H, s), 2.05 (3H, s), 2.11 (3H, s), 2.25 (1H, dd, J=13.1, 6.1 Hz), 2.51 (1H, sext, J=6.9 Hz), 2.85 (1H, dd, J=14.1, 3.9 Hz), 2.97 (1H, dd, J=10.8, 6.1 Hz), 3.70 (1H, m), 3.72 (1H, s), 4.17 (1H, s), 4.33 (1H, d, J=13.5 Hz), 4.43 (1H, d, J=13.5 Hz), 5.44 (1H, d, J=4.4 Hz), 5.46 (1H, s), 5.69 (1H, d, J=4.4 Hz), 5.81 (1H, s), 5.97 (1H, t, J=4.4 Hz), 6.09 (1H, m), 7.12 (1H, br s), 7.34 (1H, t, J=1.5 Hz); SIMS m/z 759 $(M+1)^+$.

Trichilin D (8). $C_{35}H_{46}O_{12}$; $[\alpha]_D^{22}$ $-73°$ (c 0.2); UV 210 nm (ε 3200); CD 206 ($\Delta\varepsilon$ −12), 293 nm ($\Delta\varepsilon$ −14); ^{1}H NMR δ=0.84 (3H, s), 0.94 (3H, t, J=7.6 Hz), 1. 09 (3H, s), 1.19 (3H, d, J=7.0 Hz), 1.24 (3H, s), 1.55 (1H, m), 1.72 (1H, hept, J=7.0 Hz), 1.73 (1H, dt, J=14.1, 3.8 Hz), 1.88 (1H, dd, J=13.6, 11.2 Hz), 2.02 (3H, s), 2.06 (1H, dt, J=2.3, 14.1 Hz), 2.13 (3H, s), 2.26 (1H, dd, J=13.6, 6.6 Hz), 2.38 (1H, d, J=4.4 Hz), 2.46 (2H, s), 2.51 (1H, sext, J=6.8 Hz), 2.74 (1H, dd, J=14.0, 4.5 Hz), 2.76 (1H, dd, J=11.2, 6.2 Hz), 3.69 (1H, m), 3.70 (1H, s), 4.24 (1H, t, J=3.9 Hz), 4.39 (1H, d, J=13.2 Hz), 4.48 (1H, d, J=13.2 Hz), 4.60 (1H, s), 5.52 (1H, d, J=4.2 Hz), 5.78 (1H, s), 5.89 (1H, t, J=3.9 Hz), 6.13 (1H, m), 7.14 (1H, m), 7.38 (1H, t, J=1.7 Hz); ^{13}C NMR δ=11.3q, 16.3q, 18.9q, 20.7q, 20.7q, 21.0q, 21.7q, 25.9t, 26.6t, 28.0d, 32.1t, 39.1d, 40.8s, 41.0d, 42.0s, 42.2s, 43.6s, 48.3d, 48.9t, 57.8d, 64.4t, 68.5d, 70.8d, 71.7d, 72.6s, 73.2d, 93.5d, 110.7d, 123.1s, 139.7d, 143.2d, 168.8s, 170.0s, 175.4s, 211.1s; CIMS m/z 659 $(M+1)^+$.

Meliatoxin A_2 (9). $C_{34}H_{44}O_{12}$; $[\alpha]_D^{22}$ $-72.5°$ (c 0.12);

UV 213 nm (ε 2500); CD 229 ($\Delta\varepsilon$ +7), 298 nm ($\Delta\varepsilon$ −39); ^{1}H NMR δ=0.84 (3H, s), 1.09 (3H, s), 1.21 (3H, d, J=7.0 Hz), 1.22 (3H, d, J=7.0 Hz), 1.24 (3H, s), 1.74 (1H, dt, J=14.5, 3.6 Hz), 1.88 (1H, dd, J=13.4, 11.0 Hz), 2.02 (3H, s), 2.05 (1H, dt, J=2.2, 14.7 Hz), 2.13 (3H, s), 2.26 (1H, dd, J=13.2, 6.6 Hz), 2.39 (1H, d, J=4.0 Hz), 2.46 (2H, s), 2.67 (1H, hept, J=7.0 Hz), 2.74 (1H, dd, J=14.5, 4.4 Hz), 2.76 (1H, dd, J=11.0, 6.0 Hz), 3.69 (1H, m), 3.71 (1H, s), 4.25 (1H, t, J=4.0 Hz), 4.39 (1H, d, J=13.4 Hz), 4.49 (1H, d, J=13.4 Hz), 4.60 (1H, s), 5.53 (1H, d, J=4.0 Hz), 5.77 (1H, s), 5.92 (1H, t, J=4.6 Hz), 6.13 (1H, m), 7.14 (1H, br s), 7.37 (1H, t, J=1.5 Hz); ^{13}C NMR δ=18.7q, 18.7q, 18.8q, 20.7q, 20.7q, 20.9q, 21.7q, 25.9t, 28.0d, 32.1t, 34.1d, 39.1d, 40.9s, 42.0s, 42.3s, 43.6s, 48.3d, 48.5t, 57.8d, 64.6t, 68.5d, 70.8d, 71.7d, 72.6s, 73.2d, 93.5d, 110.7d, 123.1s, 139.7d, 143.2d, 168.8s, 170.0s, 175.8s, 211.1s; SIMS m/z 645 $(M+1)^+$.

Antifeedant Activities. All of the isolated limonoids, **1**—**9**, were tested by the conventional leaf disk method[12] against the larvae of *Spodoptera exigua* Hübner (Boisduval). *Effective conc.*: azedarachin A (**1**), trichilin B (**4**); 200 ppm. 12-*O*-Acetylazedarachin A (**2**), 12-*O*-acetylazedarachin B (**3**), trichilin H (**5**), 12-*O*-acetyltrichilin B (**6**), 1,12-di-*O*-acetyltrichilin B (**7**), trichilin D (**8**), meliatoxin A_2 (**9**); 400 ppm.

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References

1) D. E. U. Ekong, C. O. Fakunle, A. K. Fasina, and J. I. Okogun, *J. Chem. Soc., Chem. Commun.*, **1969**, 1166.

2) E. D. Morgan and M. D. Thornton, *Phytochemistry*, **12**, 391 (1973).

3) S. M. Lee, J. A. Klocke, and M. F. Balandrin, *Tetrahedron Lett.*, **28**, 3543 (1987).

4) P. B. Oelrichs, M. W. Hill, P. J. Vallely, J. K. Macleod, and T. F. Molinski, *Phytochemistry*, **22**, 531 (1983).

5) M. Ochi, H. Kotsuki, H. Ishida, and T. Tokoroyama, *Chem. Lett.*, **1978**, 99; J. D. Warthen, Jr., *Proc. Entomol. Soc. Wash.*, **91**, 367 (1989).

6) X. J-xi and Y. A-xing, *Acta Pharm. Sin.*, **20**, 188 (1985).

7) M. Ochi, H. Kotsuki, T. Kataoka, T. Tada, and T. Tokoroyama, *Chem. Lett.*, **1978**, 331.

8) M. Nakatani, S. Arikawa, H. Okamura, and T. Iwagawa, *Heterocycles*, **38**, 327 (1994).

9) M. Nakatani, R. C. Huang, H. Okamura, and T. Iwagawa, *Chem. Lett.*, **1993**, 2125.

10) Sendanin was isolated as a 29-acetate: M. Ochi, H. Kotsuki, K. Hirotsu, and T. Tokoroyama, *Tetrahedron Lett.*, **17**, 2877 (1976).

11) M. Nakatani, R. C. Huang, H. Okamura, H. Naoki, and T. Iwagawa, *Phytochemistry*, **36**, 39 (1994).

12) K. Wada and K. Munakata, *Agric. Food Chem.*, **17**, 471 (1968).

13) M. Nakatani, T. Iwashita, H. Naoki, and T. Hase, *Phytochemistry*, **24**, 195 (1985), and references cited therein.

14) M. Nakatani, J. C. James, and K. Nakanishi, *J. Am. Chem. Soc.*, **103**, 1228 (1981).

15) M. Nakatani and K. Nakanishi, *Heterocycles*, **36**, 725 (1993).

16) J. Polonsky, Z. Varon, B. Arnoux, and C. Poscard, *J. Am. Chem. Soc.*, **100**, 2575 (1981); M. Nakatani and K. Nakanishi, *The Reports of the Faculty of Science, Kagoshima University*, **25**, 59 (1992).

17) S. V. Ley, A. A. Denholm, and A. Wood, *Nat. Prod. Rep.*, **1993**, 109.

18) D. E. Champagne, O. Koul, M. B. Isman, G. G. E. Scudder, and G. H. N. Towers, *Phytochemistry*, **32**, 377 (1992).

19) We also isolated toosendanin (chuanliansu) as an equilibrium mixture of *exo*- and *endo*-forms from Okinawan *M. azedarach* L. and the acetylation of it with Ac_2O in pyridine afforded sendanin (**10**) in a high yield: M. Nakatani, R. C. Huang, S. Arikawa, K. Yamauchi, H. Okamura, T. Iwagawa, and H. Naoki, "35th Symposium on the Chemistry of Nat. Products," Kyoto, October 1993, Abstr., p. 385.