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LIMONOID ANTIFEEDANTS FROM *MELIA TOOSENDAN*

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Key Word Index—*Melia toosendan*; Meliaceae; antifeedants, trichilins; azedarachins.

Abstract—Two new insect antifeeding limonoids, trichilins K and L, were isolated from the stem bark of *Melia toosendan* along with five known limonoids, trichilins H, I and J, azedarachin A and 12-*O*-acetyl-azedarachin B. Their structures were elucidated by spectroscopic means and their antifeeding properties were examined with the larvae of *Spodoptera eridania*.

INTRODUCTION

Meliaceae plants are a rich source of limonoids. A typical plant *Melia azedarach* Linn. naturalized on a number of continents in the world, is of particular interest, because it contains many limonoids showing antifeedant activity. We have studied the bioactive limonoids of the plants collected at Okinawa in the Ryukyu Islands and Guangzhou in China, obtained several new types of limonoids, the meliacarpinins [1, 2], trichilins [3, 4] and azedarachins [5], as insect antifeedants. We have isolated two new trichilins, K and L, from the stem bark of a related Chinese plant *Melia toosendan* Sieb. *et* Zucc. collected at Xiangtan in China along with five known limonoids, trichilins H, I and J [5, 6], azedarachin A and 12-*O*-acetylazedarachin B [5]. We report here the isolation and structures of these new limonoids and the antifeeding properties of the isolated limonoids as determined by a conventional leaf disk method [7] against the larvae of the Japanese pest insect *Spodoptera eridania* (Boisduval).

RESULTS AND DISCUSSION

The diethyl ether extract of the stem bark contained a variety of limonoids which were detected by the characteristic colour with Ehrlich's reagent on TLC. These limonoids are very sensitive to traces of acid and gradually decomposed on a silica column [8]. It was, therefore, necessary to use flash chromatography and HPLC separation techniques, and the isolation of the various congeners, **1**–**7**, was a tedious process requiring a carefully combined use of normal and reversed phase HPLC. Trichilins H (**3**), I (**4**) and J (**5**), azedarachin A (**6**) and 12-*O*-acetylazedarachin B (**7**) were identified by comparing their NMR and IR spectra with those of authentic samples.

Trichilin L (**2**) was isolated as an amorphous solid. The IR spectrum showed absorption bands of hydroxyl groups (3452 cm^{-1}) and two different carbonyl groups of esters (1740 cm^{-1}) and a ketone (1710 cm^{-1}). The SI-mass spectrum showed pseudo-molecular ions at *m/z* 639 $[M + Na]^+$ and 617 $[M + 1]^+$. The structure of **2** was confirmed by extensive ^1H and ^{13}C NMR studies, including COSY, DEPT spectra and NOE experiments, and circular dichroism (CD) data ($\Delta\varepsilon_{303}$ − 5.1; n − π^* of 11-oxo group), and their comparison with the corresponding data of the known trichilins. The ^1H and ^{13}C NMR data (27 and 45°C) indicated that **2** contained six methyls, five methylenes, thirteen methines, nine carbons (one ketone and two acyloxy) not bonded to hydrogen and three protons due to hydroxyl groups. The ^1H NMR spectrum revealed the presence of the typical 2-methylbutanoyl and acetyl substituents and a 3-furyl moiety.

The ^1H and ^{13}C NMR spectra indicated the presence of the 14,15-epoxide [δ3.68 (*s*, H-15α), and δ58.0 *d* (C-15) and 72.7 *s* (C-14)] and the 19/29 bridged acyl acetal ester system [δ4.37 and 4.41 (each *d*, J = 12.9 Hz, H_2-19) and 5.78 (*s*, H-29), and δ64.3 *t* (C-19) and 94.2 *d* (C-29)] like trichilin H (**3**) [6]. Irradiation of the 8- (δ1.07) and 13-Me (δ1.20) peaks enhanced one signal (δ4.41) of 19-H_2 and the H-7 (δ3.68) signal, and the H-9 (δ4.59), H-21 (δ7.11) and H-22 (δ6.10) signals, respectively. Another peak of 19-H_2 at δ4.37 showed a W-type long range coupling with the H-5 signal at δ2.68, and the H-1 and H-9 signals at δ4.29 and 4.59 also showed long range couplings with the 3-H and 8-Me signals at δ4.11 and 1.07, respectively. The 12-methylene protons were observed as a singlet at δ2.42 like trichilins D and J (**5**) [6]. The substitution pattern around the A-ring, namely, that **2** has free 1α, 3α-OH and 2α-acetoxyl groups, was assigned from the

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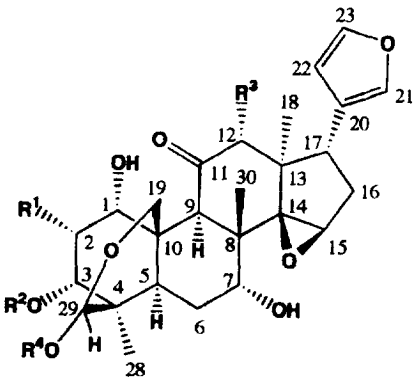

	R^1	R^2	R^3	R^4
1:	OH	Ac	H	$COCH(CH_3)_2$
2:	OAc	H	H	$COCH(CH_3)CH_2CH_3$
3:	OAc	Ac	OAc	$COCH(CH_3)_2$
4:	OH	Ac	OAc	$COCH(CH_3)CH_2CH_3$
5:	OH	Ac	H	$COCH(CH_3)CH_2CH_3$
6:	H	Ac	OH	$COCH(CH_3)CH_2CH_3$
7:	H	Ac	OAc	$COCH(CH_3)_2$

couplings between H-1β, 2β and 3β signals and the fact that the H-9 signal in **2** was at δ4.59, due to the effect of the 1α-hydroxyl in a 1,3-diaxial relationship; δ4.66 and 4.59 in **4** and **5**; in the 1-*O*-acetylated compounds, it was observed at δ4.0–4.2 [9–11].

It is apparent that the low shift of the H-1β signal at δ4.29 was caused by an anisotropic effect of the 11-keto group, compared to δ3.94 in trichilin C (12-oxo compound) [Nakatani, M.; unpublished data]. On the other hand, the exo-configuration of the 2-methylbutanoyl group was established from the low chemical shift of the H-3 signal at δ4.11, as well as that in acetyl migrated trichilins [10] which have been correlated to aphanastatin [9] determined by X-ray analysis. These data strongly suggested that **2** was 3-deacetyltrichilin D.

A ^{1}H NMR study of trichilin K (**1**) together with the pseudo-molecular ion peaks of *m/z* 625 $[M + Na]^+$ and 603 $[M + H]^+$ by SI-mass spectrum, gave $C_{32}H_{42}O_{11}$. The CD spectrum showed the presence of 11-oxo group at 302 nm ($\Delta\varepsilon$ − 10) and the ^{1}H NMR spectrum was very similar to that of **2**, except that the ester moiety at C-29 was 2-methylpropanoyl and the 2-acetyl group in **2** was migrated to C-3 in **1**. The substitution pattern around the A-ring, i.e. **1** has free 1,2-dihydroxyl groups and a 3-acetyl group, as in trichilin I (**4**), was also shown by the fact that the H-9 signal was at δ4.59 due to the effect of the

Table 1. ^{1}H NMR data of compounds **1**–**3** and **6** (400 MHz, $CDCl_3$)

H	1	2	3	6
1	4.20 *d* (*br*) (4.8)	4.29 *m*	4.42 *t* (4.2)	4.47 *m*
2	4.70 *t* (4.8)	5.76 *t* (4.3)	5.90 *t* (*br*) (4.4)	1.89 *d* (*br*) (16.0) 2.86 *dt* (16.3, 4.9)
3	5.43 *d* (4.8)	4.11 *m*	5.53 *d* (4.5)	5.33 *d* (*br*) (4.6)
5	2.71 *dd* (13.8, 3.8)	2.68 *dd* (13.6, 3.9)	2.81 *dd* (13.3, 3.7)	2.72 *dd* (14.0, 4.0)
6α	1.73 *dt* (14.6, 3.9)	1.72 *dt* (14.3, 3.6)	1.74 *dt* (14.3, 3.6)	1.73 *dt* (14.3, 3.7)
6β	2.04 *dt* (2.3, 14.6)	2.07 *dt* (2.2, 14.3)	2.03 *m*	2.06 *dt* (2.0, 14.0)
7	3.69 *m*	3.68 *s* (*br*)	3.68 *m*	3.66 *m*
9	4.59 *s*	4.59 *s*	4.64 *s*	4.52 *s*
12	2.47 *s*	2.42 *s*	5.40 *s*	4.11 *s*
15	3.70 *s*	3.68 *s*	3.76 *s*	3.77 *s*
16α	2.26 *ddd* (13.4, 6.2, 0.6)	2.23 *ddd* (13.4, 5.9, 1.1)	2.24 *dd* (13.1, 6.1)	2.35 *dd* (13.4, 6.4)
16β	1.88 *dd* (13.6, 11.4)	1.86 *dd* (13.0, 11.0)	1.90 *dd* (13.3, 11.4)	1.91 *dd* (13.0, 11.0)
17	2.76 *dd* (10.9, 6.2)	2.72 *dd* (11.0, 6.3)	2.99 *dd* (11.3, 6.1)	3.02 *dd* (11.4, 6.2)
18 (Me)	1.26 *s*	1.20 *s*	1.35 *s*	1.17 *s*
19a	4.38 *d* (13.2)	4.37 *d* (12.9)	4.31 *d* (13.6)	4.26 *d* (12.3)
19b	4.55 *d* (13.2)	4.41 *d* (12.9)	4.38 *d* (13.6)	4.32 *d* (12.3)
21	7.14 *m*	7.11 *m*	7.13 *m*	7.22 *m*
22	6.14 *m*	6.10 *m*	6.12 *m*	6.53 *m*
23	7.37 *t* (1.5)	7.35 *t* (1.5)	7.34 *t* (1.4)	7.32 *t* (1.5)
28 (Me)	0.82 *s*	0.98 *s*	0.84 *s*	0.82 *s*
29	5.72 *s*	5.78 *s*	5.75 *s*	5.80 *s*
30 (Me)	1.08 *s*	1.07 *s*	1.17 *s*	1.14 *s*
2′	2.62 *hept* (7.0)	2.44 *hext* (7.0)	2.67 *hept* (7.0)	2.45 *hext* (7.0)
2′-Me	1.19 *d* (7.0)	1.15 *d* (7.0)	1.21 *d* (7.0)	1.17 *d* (7.0)
3′a	1.20 *d* (7.0)	1.66 *m*	1.22 *d* (7.0)	1.71 *hept* (7.0)
3′b	—	1.50 *m*	—	1.53 *hept* (7.0)
3′-Me	—	0.90 *t* (7.0)	—	0.93 *t* (7.7)
Ac (2α)	—	2.12 *s*	2.02 *s*	—
(3α)	2.14 *s*	—	2.13 *s*	2.11 *s*
(12α)	—	—	1.98 *s*	—

Table 2. ^{13}C NMR data of compounds **2**, **3**, **6** and **7** (100 MHz, $CDCl_3$)

C	2	3	6	7
1	73.1 *d*	71.6 *d*	70.6 *d*	70.1 *d*
2	71.0 *d*	68.5 *d*	33.4 *d*	33.6 *d*
3	73.5 *d*	73.0 *d*	74.0 *d*	73.6 *d*
4	39.2 *s*	40.7 *s*	39.8 *s*	39.5 *s*
5	33.7 *d*	34.1 *d*	41.4 *d*	34.1 *d*
6	26.8 *t*	25.6 *t*	25.7 *t*	25.8 *t*
7	71.0 *d*	70.2 *d*	70.4 *d*	70.5 *d*
8	41.2 *s*	42.5 *s*	41.9 *s*	41.5 *s*
9	48.7 *d*	48.0 *d*	48.1 *d*	48.4 *d*
10	41.2 *s*	42.3 *s*	42.5 *s*	42.5 *s*
11	205.4 *s*	206.3 *s*	207.1 *s*	206.6 *s*
12	48.6 *t*	77.9 *d*	79.2 *d*	78.6 *d*
13	43.6 *s*	45.6 *s*	46.4 *s*	45.9 *s*
14	72.7 *s*	71.9 *s*	71.4 *s*	72.0 *s*
15	58.0 *d*	58.8 *d*	59.5 *d*	58.5 *d*
16	32.0 *t*	33.8 *t*	35.7 *t*	35.0 *t*
17	39.2 *d*	38.1 *d*	38.9 *d*	38.3 *d*
18	21.7 *q*	22.4 *q*	23.1 *q*	22.3 *q*
19	64.3 *t*	64.1 *t*	65.0 *t*	64.6 *t*
20	123.5 *s*	122.5 *s*	121.1 *s*	122.5 *s*
21	143.3 *d*	142.4 *d*	142.6 *d*	142.4 *d*
22	110.8 *d*	111.9 *d*	113.1 *d*	111.9 *d*
23	139.8 *d*	140.7 *d*	141.0 *d*	140.7 *d*
28	19.5 *q*	18.8 *q*	19.5 *q*	19.4 *q*
29	94.2 *d*	93.5 *d*	94.6 *d*	94.3 *d*
30	14.2 *q*	15.5 *q*	14.7 *q*	15.8 *q*
1′	175.7 *s*	175.7 *s*	175.6 *s*	175.7 *s*
2′	28.2 *d*	27.7 *d*	28.3 *d*	28.0 *d*
2′-Me	16.4 *q*	18.7 *q*	16.8 *q*	18.6 *q*
3′	22.8 *t*	18.7 *q*	26.9 *t*	18.9 *q*
3′-Me	11.5 *q*	—	11.7 *q*	—
COMe	20.8 *q*	20.7 *q*	21.8 *q*	20.7 *q*
	—	20.7 *q*	—	21.5 *q*
	—	20.9 *q*	—	—
COMe	166.6 *s*	168.9 *s*	169.7 *s*	169.8 *s*
	—	170.0 *s*	—	170.4 *s*
	—	170.2 *s*	—	—

1α-hydroxyl group. It is of interest that trichilins have been found from some species of Meliaceae plants but 2-methylpropanoyl as a C-29 ester moiety has been observed only in those from *Melia* [3, 4, 12]. The well-known acid catalysed rearrangement of the ring D epoxide to the 15-oxo structure [13] was observed in the NMR measurements of the compounds **1**, **2** and **5** in $CDCl_3$, but the other limonoids, **3**, **4**, **6** and **7**, having a 12-oxy function did not isomerize in the same condition.

The compounds, **1**–**7**, showed antifeedant activity against the larvae of a Japanese pest insect *Spodoptera eridania* (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 [7]. The activity is less than those of the azadirachtins [14] from the Indian neem tree *M. azadirachta* indica and the meriacarpinins [1, 2] from Okinawan and Chinese *M. azedarach* L., but comparable to that of trichilin B [15]. The 12-deoxy, **1**, **2** and **5**, and 12-acetoxy compounds, **3**, **4** and **7**, were active at 400 ppm. These activities are almost independent of the substitution pattern in ring A and the 28-ester moiety.

EXPERIMENTAL

^1H and ^{13}C NMR: with 400 and 100 MHz in $CDCl_3$. $[\alpha]_D$, UV and CD: in MeOH. Bioassay of the antifeedant was by the leaf disk method against the third larvae of *S. eridania*.

Plant material. The stem bark was collected in December 1992 at Xiangtan, China.

Extraction and isolation. The dried bark (530 g) was extracted with Et_2O (20 L), 15°C, 2 weeks, to yield 6.0 g of an extract, which was flash chromatographed on silica gel with 50% hexane–Et_2O, and each resulting limonoid fr. was sepd through HPLC, μ-Porasil and μ-Bondasphere columns, with 0.7–2.0% MeOH–CH_2Cl_2 and 20–40% H_2O–MeOH as the solvents, respectively, to give the following limonoids: **1** (0.8 mg), **2** (1.1 mg), **3** (0.7 mg), **4** (0.4 mg), **5** (0.9 mg), **6** (1.0 mg) and **7** (1.2 mg).

Trichilin K (**1**), $C_{32}H_{42}O_{11}$; SIMS *m/z*: 625 $[M + Na]^+$, 603 $[M + 1]^+$; $[\alpha]_D^{22}$ − 20° (*c* 0.05); UV λ_{max} nm (ε): 206.5 (3000); IR ν_{max}^{KBr} cm^{-1}: 3420, 1740, 1700, 1640, CD nm: $\Delta\varepsilon_{302}$ − 10 ($n\pi^*$ of 11-keto group). Trichilin L (**2**), $C_{33}H_{44}O_{11}$; SI-MS *m/z*: 629 $[M + Na]^+$, 617 $[M + 1]^+$; $[\alpha]_D^{22}$ − 14° (*c* 0.06); UV λ_{max} nm (ε): 212 (2100); IR ν_{max}^{KBr} cm^{-1}: 3450, 1740, 1710, 1650; CD nm: $\Delta\varepsilon_{303}$ − 5.1.

Trichilin H (**3**), $C_{36}H_{46}O_{14}$; SIMS *m/z* 703 $[M + 1]^+$; $[\alpha]_D^{22}$ − 20° (*c* 0.12); UV λ_{max} nm (ε): 213 (5500); IR ν_{max}^{KBr} cm^{-1}: 3452, 1739, 1701, 1655; CD nm: $\Delta\varepsilon_{308}$ − 2.1. Trichilin I (**4**), $C_{35}H_{46}O_{13}$; SIMS *m/z*: 675 $[M + 1]^+$; UV λ_{max} nm (ε): 211 (3200); IR ν_{max}^{KBr} cm^{-1}: 3450, 1740, 1700, 1650; CD nm: $\Delta\varepsilon_{302}$ − 3.1. Trichilin J (**5**), $C_{33}H_{44}O_{11}$; SIMS *m/z*: 617 $[M + 1]^+$; $[\alpha]_D^{23}$ + 20° (*c* 0.06); UV λ_{max} nm (ε): 206 (5500); IR ν_{max}^{KBr} cm^{-1}: 3450, 1740, 1700, 1640; CD nm: $\Delta\varepsilon_{302}$ − 5.8. Azedarachin A (**6**), $C_{33}H_{44}O_{11}$; SIMS *m/z*: 639 $[M + Na]^+$, 617 $[M + 1]^+$; $[\alpha]_D^{22}$ − 10° (*c* 0.05); UV λ_{max} nm (ε): 213 (4300); CD nm: $\Delta\varepsilon_{310}$ − 26. 12-*O*-Acetylazedarachin B (**7**), $C_{34}H_{44}O_{12}$; SIMS *m/z* 667 $[M + Na]^+$, 645 $[M + 1]^+$; $[\alpha]_D^{22}$ − 55° (*c* 0.13); UV λ_{max} nm (ε): 213 (3000); CD nm: $\Delta\varepsilon_{308}$ − 10.

Bioassay of the antifeedants. The antifeedant potential of the compounds was assessed by presenting them on leaf disks of a Chinese cabbage to the third instar larvae of *Spodoptera eridania* (Boisduval) and visually comparing the treated and untreated leaves eaten by the larvae. The larvae were placed in a Petri dish with the five treated leaf disks with sample and the five untreated disks as controls. The feeding bioassays terminated after the larvae had eaten approximately 50% of one of the disks, which took 6–24 hr. This choice test was done at 100, 200, 300, 400 and 500 ppm concentrations to determine minimum inhibitory concentration for each of the compounds.

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REFERENCES

1. Nakatani, M., Arikawa, S., Okamura, H. and Iwagawa, T. (1994) *Heterocycles* **38**, 327.
2. Nakatani, M., Huang, R. C., Okamura, H. and Iwagawa, T. (1993) *Chem. Lett.* 2125.
3. Nakatani, M., Huang, R. C., Okamura, H., Naoki, H. and Iwagawa, T. (1994) *Phytochemistry* **36**, 39.
4. Huang, R. C., Okamura, H., Iwagawa, T., Tadera, K. and Nakatani, M. *Phytochemistry*, in press.
5. Huang, R. C., Okamura, H., Iwagawa, T. and Nakatani, M. (1994) *Bull. Chem. Soc. Jpn.* **67**, 2468.
6. Nakatani, M., Zhou, J-Bo, Iwagawa, T. and Okamura, H. (1994) *Heterocycles* **38**, 2407.
7. Wada, K. and Munakata, K. (1968) *Agric. Food Chem.* **17**, 471.
8. Nakatani, M., Iwashita, T., Naoki, H. and Hase, T. (1985) *Phytochemistry* **24**, 195, and references cited therein.
9. Polonsky, J., Varon, Z., Arnoux, B. and Poscard, C. (1981) *J. Am. Chem. Soc.* **100**, 2575.
10. Nakatani, M. and Nakanishi, K. (1992) *The Reports of the Faculty of Science, Kagoshima University, (Math., Phys. & Chem.)* **25**, 59.
11. Nakatani, M. and Nakanishi, K. (1993) *Heterocycles* **36**, 725.
12. Oelrichs, P. B., Hill, M. W., Vallely, P. J., McLeod, J. K. and Molinski, T. F. *Phytochemistry* **22**, 531.
13. Chan, W. R., Gibbs, J. A. and Taylor, D. R. (1967) *J. Chem. Soc., Chem. Commun.* 720.
14. Ley, S. V., Denholm, A. A. and Wood, A. (1993) *Natural Product Reports*, 109.
15. Champagne, D. E., Koul, O., Isman, M. B., Scudder, G. G. E. and Towers, G. H. N. (1992) *Phytochemistry* **31**, 377.