ISOLATION OF TWO LIMONOID ANTIFEEDANTS FROM *MELIA TOOSENDAN*

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Abstract — Two new limonoid antifeedants, trichilins I and J, were isolated from the stem bark of *Melia toosendan* (Meliaceae) and their structures were elucidated by spectroscopic means.

Meliaceae plants are attracting conciderable interest, particulary because they contain several types of limonoids showing antifeedant activities; intact apoeuphol skeleton limonoids,[1-3] A,B-ring seco,[4,5] B-ring seco[6] and C-ring seco limonoids.[2,7] Many studies have been reported on the constituents of a typical plant *Melia azedarach* L.[8] We have also studied on the limonoid antifeedants from the Okinawan and Chinese plants to isolate three different type compounds; meliacarpinins,[9] trichilins[10] and azedarachins.[11] Upon continued research, we isolated two new limonoids, trichilins I and J, and three known limonoids as insect antifeedant from a related tree *M.toosendan* Sieb. *et* Zucc. collected at Xiangtan in China. We report the isolation and structures of these new trichilins.

Two new trichilins, I (**1**; 0.4 mg) and J (**2**; 0.9 mg), were isolated along with three known limonoids, trichilin H (**3**; 0.7 mg),[10] azedarachin A (**4**; 1.0 mg)[11] and 12-*O*-acetylazedarachin B (**5**; 1.2 mg),[11] from the ether extract of the dried stem bark (530 g) by a carefully combined use of normal and reversed phase hplc. Since the antifeeding limonoids were very sensitive to traces of acid and gradually decomposed, it was necessary to use flash chromatography and hplc for the separation of the various congeners (**1**-**5**).

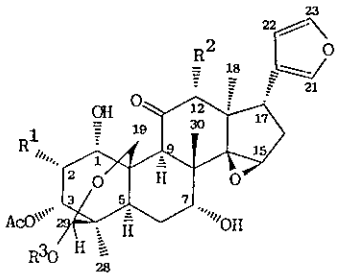
The molecular formula $C_{35}H_{46}O_{13}$ of **1** was derived from the SIms (m/z 675 $[M+1]^+$) and ^1H nmr data (Table 1). Taking into account the cd ($\Delta\varepsilon_{302}$ -3.1; n-π^* of 11-oxo group) and ir

data (3450, 1740 and 1700 cm^{-1}), the ^1H nmr studies including $^1H-^1H$ COSY and nOe experiments allowed us to predict (**1**) to be 2-deacetyl-12-*O*-acetyltrichilin B. The ^1H nmr spectrum was very similar to that of trichilin B,[1] including the signals due to the 14,15-epoxy [δ 3.74(15-H)] and 19/29 bridged acyl acetal ester [δ 4.33, 4.37(19-H_2) and 5.72(29-H)] and two acetyl and one 2-methylbutanoyl groups (Table 1), except for some differences of chemical shifts. The substitution pattern around the A-ring, i.e. **1** has a free 1α,2α-dihydroxyl group, the same as trichilin G,[12] was shown by the fact that the 9-H signal in **1** was at δ 4.66 due to the effect of the 1α-hydroxyl in a 1,3-diaxial relationship. On the other hand, the fact that the 12-OAc in **1** is α, was deduced from the chemical shifts of the 1β- and 17-H at δ 4.32 and 2.97, which were shifted to δ 4.06 and 3.16 in 12-*O*-acetyltrichilin A (12β-OAc).[13] The stereochemistry of **1** was confirmed by nOe enhancements between 8-Me peak and the 7-H and one of the 19-H_2 signals and the 13-Me peak and the 9-, 21- and 22-H signals, and long range couplings between the another peak of the 19-H_2 and the 5-H signal, the 1- and 3-H signals and the 9-H and 8-Me signals.

The second limonoid, trichilin J (**2**), $C_{33}H_{44}O_{11}$, SIms (m/z 617 $[M+1]^+$) was also showed the presence of the 11-oxo (cd: $\Delta\varepsilon_{305}$ -5.8 and ir: 1700 cm^{-1}) and ester groups (ir: 1740 cm^{-1}). The ^1H nmr spectrum was superimposable on that of **1** except for the luck of one acetoxyl group and some differences of chemical shifts (Table 1). The fact that the 12-OAc in **1** is missing in **2**, was readily deduced from a 2H-singlet at δ 2.47. With the luck of the 12-OAc group, the 1- and 17-H signals at δ 4.32 and 2.97 in **1** changed to δ 4.19 and 2.75 in **2**, respectively, and these changes could be attributed to the conformational change of the ring C in **2**.

The *exo*-configuration of the 2-methylbutanoyloxy group at C-29 (*S*) in **1** and **2** has been established from the chemical shifts of the 3-H signals which appeared at the low positions of δ 5.47 and 5.43, as well as all of known trichilins and azedarachins, compared to δ 4.90 and 5.07 in the *endo*-isomers of toosendanin[11] and its 29-*O*-benzoate.[14]

The compounds (**1**-**5**) showed antifeedant activity against the larvae of Japanese pest insect *Spodoptera exigua* Hübner (Boisduval). The most potent is azedarachin A (**4**) with a 12-OH function, which is active at 200 ppm, corresponding to the concentration of ca 4



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

Table 1. ^{1}H Nmr data for trichilins I (1) and J (2) (400 MHz, $CDCl_3$)

H	1 δ Mult(J/Hz)	2 δ Mult(J/Hz)	H	1 δ Mult(J/Hz)	2 δ Mult(J/Hz)
1	4.32 m	4.19 d(4.4)	19a	4.33 d(13.6)	4.37 d(13.3)
2	4.64 t(4.6)	4.71 t(4.7)	19b	4.43 d(13.6)	4.54 d(13.3)
3	5.47 d(4.7)	5.43 d(4.7)	21	7.14 m	7.14 m
5	2.75 dd(13.6, 3.7)	2.71 dd(15.1, 4.1)	22	6.18 br s	6.14 m
6	1.71 br d(15.3)	1.73 br d(15.5)	23	7.34 t(1.4)	7.37 t(1.7)
6	2.04 br t(13.6)	2.04 dt(2.2, 13.8)	28(Me)	0.81 s	0.82 s
7	3.68 m	3.68 br dd(2.7, 2.2)	29	5.72 s	5.73 s
9	4.66 s	4.59 s	30(Me)	1.13 s	1.08 s
12	5.10 s	2.47 s	2'	2.46 sext(7.0)	2.45 sext(7.0)
15	3.74 s	3.70 s	2'-Me	1.18 d(7.0)	1.17 d(7.0)
16	2.25 dd(13.5, 7.2)	2.26 dd(13.3, 11.3)	3'	1.54 m	1.50 m
16	1.94 dd(13.6, 11.5)	1.88 dd(13.3, 11.3)		1.70 m	1.70 m
17	2.97 dd(11.2, 6.7)	2.75 dd(11.1, 6.1)	3'-Me	0.93 t(7.0)	0.93 t(7.3)
18(Me)	1.30 s	1.26 s	Ac	1.99 s, 2.14 s	2.14 s

$\mu g/cm^2$, by the conventional leaf disk method.[15] Then, the 12-acetoxy (**1**, **3** and **5**) and the 12-deacetoxy compounds (**2**) were active at 400 ppm. These activities were almost independent of the substitution pattern in ring A and the 29-ester moiety.

EXPERIMENTAL

^{1}H Nmr and ^{1}H-^{1}H COSY spectra were measured in $CDCl_3$ on a JEOL FX-400 spectrometer. Ir and uv spectra were recorded in $CDCl_3$ and MeOH on JASCO FT/IR 5300 and Shimadzu UV-210 A spectrophotometers. Optical rotation and cd spectra were measured using a JASCO J-20 A 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, respectively.

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

Extraction and isolation. The dried stem bark (530 g) was extracted with ether (20 l), 15°C, 2 weeks, to yield 6 g of an extract, which was flash chromatographed on SiO_2 (150 g) with 50 % hexane-ether, and each resulting limonoid fraction was separated through hplc using normal and reversed columns to give following limonoids : 1 (0.4 mg), **2** (0.9 mg), **3** (0.7 mg), 4 (1.0 mg) and 5 (1.2 mg).

Trichilin I (1). An amorphous powder, $C_{35}H_{46}O_{13}$; uv 211 nm (ε 3200); ir 3450, 1740, 1700 and 1650 cm^{-1}; cd 302 nm (Δε -3.1); SIms m/z 675 $[M+1]^+$.

Trichilin J (2). An amorphous powder, $C_{33}H_{44}O_{11}$; $[\alpha]_D^{23}$ +20° (c 0.06); uv 206 nm (ε 5500); ir 3450, 1740, 1700 and 1640 cm^{-1}; cd 302 nm (Δε -5.8); SIms m/z 617 $[M+1]^+$.

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