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Limonoid Antifeedants from *Melia toosendan* (Meliaceae)

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Abstract

Ten new limonoids have been isolated as insect antifeedants along with seven known limonoids from the stem and root bark of *Melia toosendan* (Meliaceae).

Introduction

Meliaceae plants are a rich source of limonoids. A popular plant *Melia azedarach* L., as well as *M. azadirachta* indica, is attracting considerable interest, particularly because of their biologically active limonoid constituents, in which an insect antifeeding property have been well studied [1]. We have also reported the structures of some new limonoid antifeedants, meliacarpinins [2–4], azedarachins [5, 6] and trichilins [7], and insect antifeedant activity of the isolated limonoids [8].

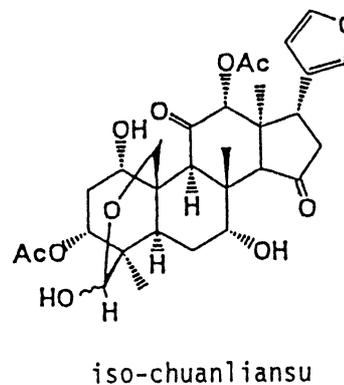
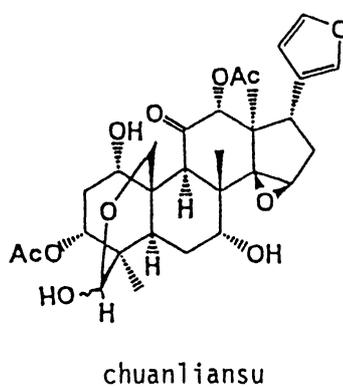
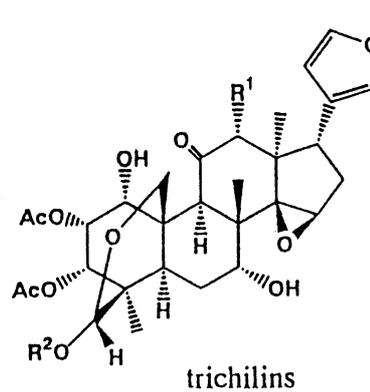
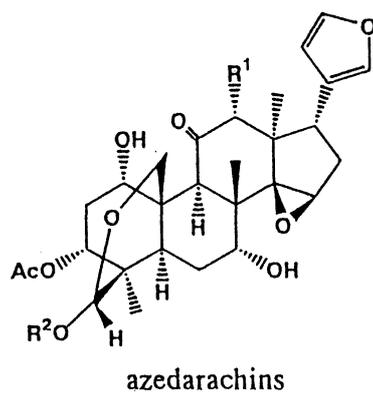
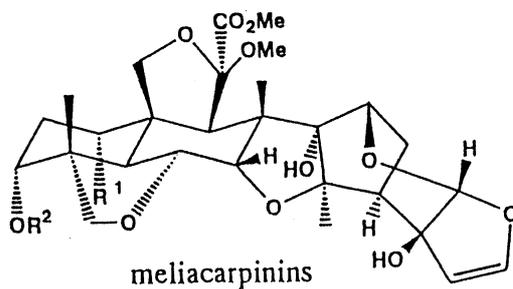
In the continuous studies on limonoid antifeedants from Meliaceae plants, we isolated ten new limonoids along with eight known compounds from the stem and root bark of *M. toosendan* collected at Xiangtan in China. As *M. toosendan* is a closely related plant to *M. azedarach*, similar limonoids, containing the same compounds, to azedarachins and trichilins from *M. azedarach* were isolated from *M. toosendan*. In addition, some different types of nimboldins and trichilinins were also isolated, but any meliacarpinin has not been observed.

In China, an extract of the bark is used as an anthelmintic and two limonoids of chuanliansu and *iso*-chuanliansu have been isolated from the bark collected at Sichuan province [9].

In this study, we isolated four new 19/29 bridged acyl acetals, trichilins I (1), J (2), K (3) and L (4), along with five known limonoids, 5–9, from the stem bark, and four new salannin-type C-seco limonoids, nimboldins C (11)–F (14) and two new intact apo-euphol limonoids, trichilinins B (16) and C (17) along with known nimboldin B (15), salannin (18) and 12-*O*-

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acetylazedarachin A (10) from the root bark. The structures of the isolated compounds were elucidated by spectroscopic and chemical means.

All of the limonoids from *M. toosendan* showed antifeedant activity against the third instar larvae of a Japanese pest insect *Spodoptera eridania* (Boisduval).

Results and Discussion

The presence of limonoids in the extract was detected by the characteristic color with Ehrlich's reagent on TLC. The antifeedant limonoids from *M. toosendan* were also very sensitive to traces of acid and gradually decomposed on a silica column. It is, therefore, necessary to use flash chromatography, p-TLC and HPLC separation techniques, and the isolation of the various congeners was a tedious process requiring a carefully combined use of normal and reversed phase HPLC. The yields of the compounds isolated from the ether extracts of the stem and root bark were follows. From the stem bark (530 g): **1** (0.4 mg), **2** (0.9 mg), **3** (0.8 mg), **4** (1.2 mg), **5** (0.7 mg), **6** (16.8 mg), **7** (4.0 mg), **8** (1.0 mg) and **9** (1.2 mg). From the root bark (1.5 kg): **10** (2.8 mg), **11** (6.6 mg), **12** (5.0 mg), **13** (4.4 mg), **14** (0.9 mg), **15** (1.5 mg), **16** (3.8 mg), **17** (0.9 mg) and **18** (4.2 mg).

1. Structures of the limonoid antifeedants

All of the known limonoids were identified by comparing their NMR and IR spectra with those of authentic samples.

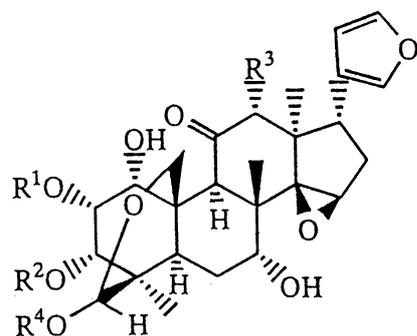
Trichilins (1–5) [10, 11]

Trichilin I (**1**) was isolated as an amorphous solid. The molecular formula $C_{35}H_{46}O_{13}$ of **1** was derived from the SI-MS (m/z 675 $[M+1]^+$) and 1H NMR data. Taking into account the CD ($\Delta\epsilon_{302} -3.1$; $n-\pi^*$ 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 [12], isolated from an African medicinal Meliaceae plant *Trichilia roka*, including the signals due to 14,15-epoxide and 19/29 bridged acyl acetal ester and two acetyl and one 2-methylbutanoyl groups, except for some differences of chemical shifts. The substitution pattern around the A-ring was same with that of trichilin G [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.

All of the other new trichilins J (**2**); $C_{33}H_{44}O_{11}$, K (**3**); $C_{32}H_{42}O_{11}$ and L (**4**); $C_{33}H_{44}O_{11}$, also showed the presence of the 11-oxo, 14,15-epoxy and 19/29 bridged acyl acetal groups. Their 1H NMR spectra were superimposable on that of **1** except for some changes of ester moieties. The 1H NMR studies of decoupling, COSY and NOE experiments allowed us to predict their structures to be **2–4**.

The *exo*-configuration at C-29 in **1–4** has been also established from the chemical shifts of the 3-H signals which appeared at lower positions compared to those in the *endo*-isomers of toosendanin and its 29-*O*-benzoate [5].

Compound **5** was identified as trichilin H by a direct comparison with an authentic sample.

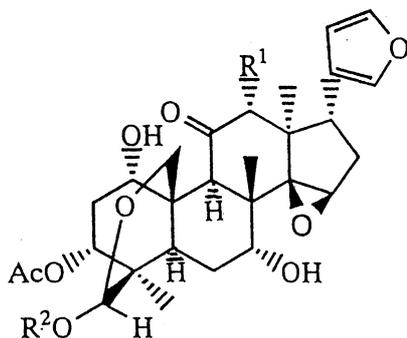


	R ¹	R ²	R ³	R ⁴
1:	H	Ac	OAc	X
2:	H	Ac	H	X
3:	H	Ac	H	Y
4:	Ac	H	H	X
5:	Ac	Ac	OAc	Y

trichilins X=COCH(CH₃)CH₂CH₃
Y=COCH(CH₃)₂

Azedarachins (6–10)

All of the azedarachin-type limonoids 6–10, containing toosendanins (6) and (7), from *M. toosendan* were known compounds already isolated by us from Chinese *M. azedarach* L. [5, 6].



	R ¹	R ²
6:	OAc	H (and 29-epimer)
7:	OH	H (and 29-epimer)
8:	OH	COCH(CH ₃)CH ₂ CH ₃
9:	OAc	COCH(CH ₃) ₂
10:	OAc	COCH(CH ₃)CH ₂ CH ₃

azedarachins

Nimboldins (11–15) and salannin (18) [14]

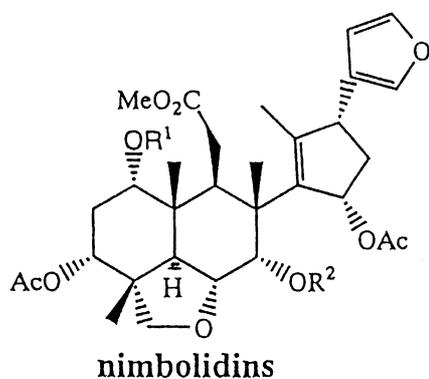
Four new limonoids, nimboldins C (11), D (12), E (13) and F (14), were isolated along with two known limonoids, nimboldin B (15) [15] and salannin (18) [16].

Nimboldin C (11) showed the presence of estercarbonyls and double bonds in the IR spectrum. The ¹³C NMR and MS data revealed the molecular formula as C₃₇H₅₀O₁₂ (13 unsaturations). The ¹³C and ¹H (at 27° and 45°C) NMR spectra indicated that 11 contained 10 CH₃, 4 CH₂, 12 CH, 11 carbons (5 carbonyls) not bonded to hydrogen, including one tetrasubstituted double bond, and no proton due to OH group. The ¹H NMR revealed the presence of a typical 2-methylpropanoyl and three acetoxyl substituents and a 3-furyl moiety. Additional presence of carbomethoxy and olefinic methyl groups and a characteristic

28-methylene group forming an ether linkage with C-6 strongly suggested that **11** was a salannin-type ring C-seco limonoid.

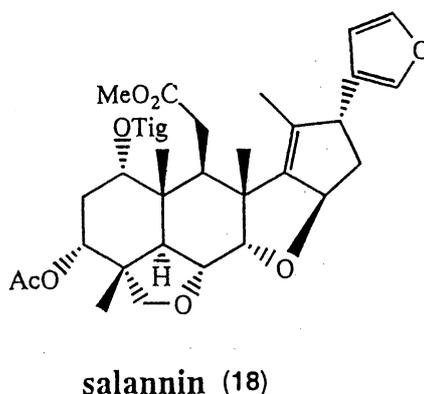
These NMR data of **11** were superimposable on that of nimboldin B (**15**), isolated from *M. azedarach* from Jugoslavian [15], and extensive NMR studies allowed us to derive the structure **11**, in which 2-methylpropanoyl was assigned at C-7 by NOEs between one 2'-methyl signal and the 13-Me signal and the other 2'-Me signal and the 15-H signal.

The structures of another nimboldins D; $C_{41}H_{54}O_{12}$, E; $C_{40}H_{54}O_{12}$ and F; $C_{41}H_{56}O_{12}$ were readily elucidated to be **12**, **13** and **14** from their similar 1H NMR spectra to **11** and **15** except for some changes of acyl substituents, in which the positions of tigloyl substitution were reduced from the low chemical shifts of the proton attached to the carbon possessing tigloyloxy group.



	R^1	R^2
11:	Ac	$COCH(CH_3)_2$
12:	Tig	Tig
13:	Tig	$COCH(CH_3)_2$
14:	$COCH(CH_3)CH_2CH_3$	Tig
15:	Ac	Tig

Salannin (**18**) is a popular C-seco limonoid isolated from many Meliaceae plants.



Trichilins (16) and (17) [17]

Trichilin B (16) exhibited the presence of a hydroxyl, saturated and unsaturated ester carbonyls and a C=C double bond except for a furan ring in the IR and UV spectra. The MS and ^{13}C NMR data revealed the molecular formula as $\text{C}_{35}\text{H}_{46}\text{O}_9$ (13 unsaturations). In the ^1H NMR spectrum, the presence of four tertiary methyls and a β -furyl moiety were observed along with a trisubstituted olefinic proton signal and a typical AB quartet due to 28-methylene protons, weakly coupled to 4 β -Me. One C-7 methine proton under hydroxyl, coupling with a methine proton at C-6 linked to an ether oxygen, was also observed along with three protons under acyloxy groups.

These data strongly suggested that 16 was a hexacyclic structure similar to vilasinin [18] and trichilin [19], and the ^1H NMR spectrum of 16 was very similar to that of trichilin except for the addition of one tigloyl group. The substitution pattern around the A-ring with 1 α -tigloyloxy and 3 α -acetoxy groups, was deduced from the long range coupling between 1 β - and 3 β -H signals and the high field shift of the 11 α -H signal, attributable to a shielding effect of the 1 α -tigloyl group [17]. Finally, stereochemistry of 16 was confirmed by NOE experiments (Fig. 1).

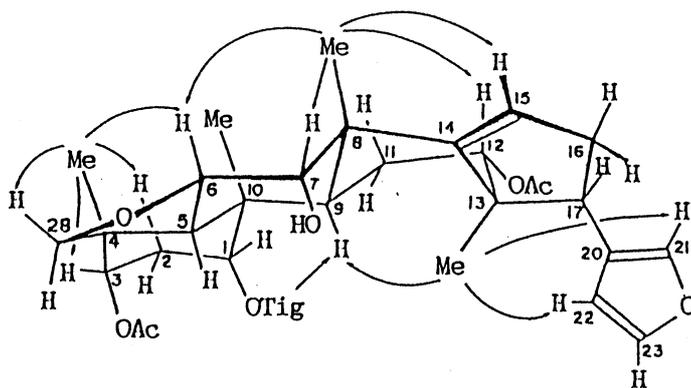
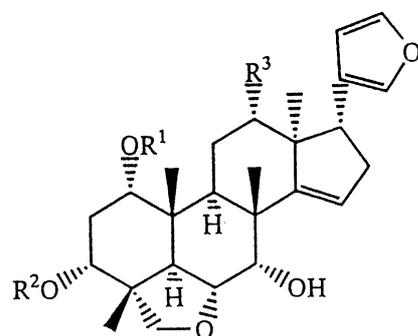


Fig. 1. Selected NOE connectivities for 16.

The ^1H NMR spectrum of trichilin C (17), $\text{C}_{33}\text{H}_{44}\text{O}_7$, was superimposable on that of 16 except for the lack of one acetoxy group. The fact that 12-OAc group is missing in 17, was apparent from the presence of 12-methylene signals and the substitution pattern of the A-ring was deduced from the large low field shifts of the 11 α - and 9-H signals.

2. Antifeedant activities of the isolated limonoids

The antifeedant activity was tested by a conventional leaf disk method [20], being assessed by presenting each test compound on leaf disks to third instar larvae of a Japanese pest insect *Spodoptera eridania* (Boisduval) and visually comparing the treated and untreated



trichilinins

	R ¹	R ²	R ³
16:	Tig	Ac	OAc
17:	Ac	Tig	H

leaves eaten by the larvae. Larvae were placed in a Petri dish with 5 leaf disks treated with a sample and 5 untreated disks as controls. The feeding bioassay was terminated after the larvae had eaten approximately 50% of these control disks, which took 6–12 h. To determine the minimum inhibitory concentration, this choice test was done at 50, 100, 150, 200, 300, 400, 500 and 1000 ppm, with 50 ppm corresponding to a concentration of ca. 1 $\mu\text{g}/\text{leaf-cm}^2$.

Table 1. Minimum inhibitory concentrations (ppm) of the isolated limonoids from *M. toosendan* in choice test with leaf disks against the third instar larvae of *S. eridania* (Boisduval).

Compound	MIC ^a (ppm)	Compound	MIC ^a (ppm)		
trichilins	1	400	azedarachin	10	400
	2	400	nimboldins	11	500
	3	400		12	500
	4	400		13	500
	5	400		14	500
azedarachins	6	300	15	500	
	7	<200	trichilinins	16	1000
	8	200		17	1000
	9	400	salannin	18	1000

^aMIC: minimum inhibitory concentration.

100 ppm is corresponding to a concentration of ca. 2 $\mu\text{g}/\text{cm}^2$.

From the antifeeding data shown in the table, it is possible to draw some general conclusions concerning the structure-activity relationships in these molecules. The activity of each tested compound was classified into two levels according to structures. The most potent were 19/29 bridged acyl acetals showed activity at 200–400 ppm. Among the bridged

acetals, the 29-hemiacetals were more active than esters. On the other hand, the activity was found to be almost independent of the substitution pattern in ring A and in the 29-ester moiety. However, the 11-keto group may be essential for their activity and 12-OH function is potent for the activity.

Salannin and related nimbolidins were apparently poor antifeedants as well as trichilinins. From these results, we believe that the potent antifeeding activity is due to certain structural features contained within the 19/29 bridged acetal fragment.

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