NIMBOLININS, C-SECO LIMONOIDS FROM THE FRUITS OF *MELIA TOOSENDAN*

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Abstract—Three new nimbolinin-type limonoids, named nimbolinins A, C and D, were isolated together with five known limonoids, trichilinin D and E, nimbolinin B, 1-*O*-cinnamoyltrichilinin and toosendanin, from the unripe fruits of a Chinese Meliaceae plant *Melia toosendan*. The structures of these new compounds were elucidated by spectrospcopic means and their antifeedant property is also described.

In a series of our experiments on limonoid constituents from Meliaceae plants, several types of compounds have been isolated as insect antifeedant from *Trichilia roka*,[1] *Melia azedarach*[2] and *Melia toosendan*.[3] The Chinaberry tree *Melia toosendan*, closely related to *M. azedarach*, has long been recognized as an insecticidal and medicinal plant in China.[4,5] Thirty seven limonoids containing nineteen new compounds have been isolated as an antifeeding constituents from the stem and root bark of *M. toosendan* collected in China.[6] From the fruits have been recently reported the isolation of five new limonoids of toosendanal (**1**), 12-*O*-methylvolkensin (**2**),[7] 1-deacetylnimbolinin A (**3**) and trichilinins D (**4**) and E (**5**).[8]

In the continuous study, three new nimbolinins, A (**6**), C (**7**) and D (**8**), were obtained from the unripe fruits collected at Xiangtan in China together with five known limonoids, trichilinin D (**4**) and E (**5**), nimbolinin B (**9**),[9] 1-*O*-cinnamoyltrichilinin (**10**)[10] and toosendanin (**11**).[2] The methylene chloride-soluble fraction of the acetone extract from the fruits was flash chromatographed on silica gel affording mixtures of limonoids,
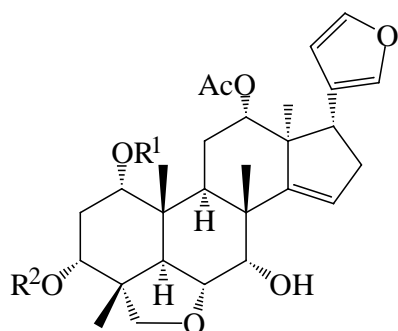
AcO

O

O

AcO

O O

AcO

H

H

H

OH

1

R³O

12

O

R¹O

9

15

1

H

13

AcO

3

7

H

OR²

O
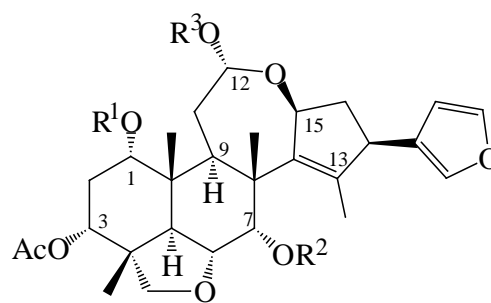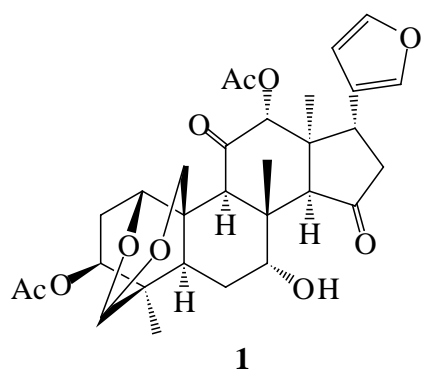
2: R¹ = Tig, R² = H, R³ = Me
3: R¹ = H, R² = Tig, R³ = H
6: R¹ = Ac, R² = Bz, R³ = H
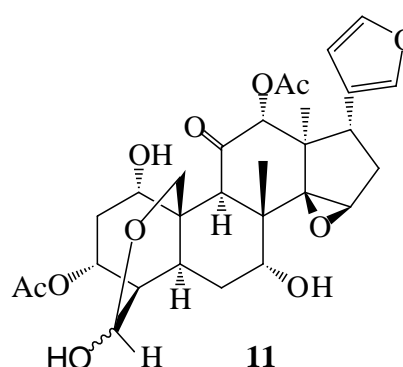**7**: R¹ = Cin, R² = H, R³ = Me
8: R¹ = H, R² = Bz, R³ = Me
9: R¹ = Ac, R² = Tig, R³ = H

AcO

OR¹

O

H

R²O

H

O

OH

4: R¹ = Cin, R² = H
5: R¹ = Bz, R² = H
10: R¹ = Cin, R² = Ac

OAc

O

OH

O

O

AcO

H

OH

HO   H   **11**

which were further purified by reversed-phase HPLC to give three new limonoids (**6**, **7** and **8**) together with five known limonoids (**4**, **5**, **9**, **10** and **11**), in which compounds (**4**, **5** and **9**) were previously isolated from the hexane-soluble part of the same specimen,[8] and the last two have been isolated from *M. azedarach*.   All of the known limonoids were identified by comparison of their NMR data with those reported.   In this paper, we report structural studies on these new limonoids and antifeedant activity of all of the isolated compounds.

Nimbolinin A (**6**) was obtained as an amorphous powder.   Its molecular formula $C_{37}H_{44}O_{10}$ (16 unsaturations) was shown by accurate MS measurement (HRFAB-MS: m/z 671.2838 [M+Na]$^+$; Δ +0.5 mmu).   The UV maxima at 217 and 228 nm and IR absorption at 3400, 1740-1710, 1660, 1600 and 1580 cm^{-1} showed the presence of carbon-carbon double bonds, hydroxyl groups and carbonyl (ester) moieties. From the ^1H and ^{13}C NMR spectra, it was evident that nine of unsaturation was present as double bonds: six carbon-carbon and three CO (esters).   Thus, the molecule is heptacyclic.   It was also clear from the NMR data that **6** contained 6 CH_3 (three tertiary, one vinylic and two acetyl), 4 CH_2, 17 CH (eight olefinic), 10 carbons (four olefinic and three ester carbonyl)) not bonded to hydrogen, and one proton due to OH group. The presence of a furan ring and two acetyl and one benzoyl groups was also apparent from the spectra.

These NMR data strongly suggested that **6** had a nimbolinin-type structure observed in the constituents from the same tree. The ^{1}H NMR spectrum is closely similar to that of nimbolinin B (**9**) isolated from *M. azedarach*,[9] in particular, with respect to the presence of a hemiacetal proton at δ 5.26 (br dt, J= 2.9 and 2.3 Hz) and an isolated AB-type methylene due to the C-6/C-28 oxymethylene bridge at δ 3.50 (d, J= 7.3 Hz, 28β-H) and 3.41 (br d, J= 7.3 Hz, 28α-H), weakly coupled to 4β-Me (29) at δ 1.20, except for the change of a tigloyl group in **9** to a benzoyl group in **6**. These data suggested that **6** differed from **9** only at C-7.

The presence of a benzoyloxy group at C-7α was deduced from the coupling (d, J= 2.6 Hz) of the downfield shifted 7β-H at δ 5.95, different from δ 5.74 in **3** and δ 5.78 in **9,** with 6β-H at δ 4.19 (dd, J= 12.9 and 2.6 Hz). A similar downfield shift for H-7 is usually observed when the bulky benzoyloxy or cinnamoyloxy groups are present at C-7α. The stereochemistry of 1– and 3-acetoxy groups was deduced from a W-type long range coupling between 1β and 3β-H at δ 4.85 (br t, J= 2.8 Hz) and δ 4.95 (br t, J= 2.8 Hz) and a NOE observation between the 3β-H and 4β-Me (29). The α-orientation for both 15-H and 12-OH was elucidated from NOE correlation between 15 and 9-H at δ 5.17 (d, J= 7.7 Hz) and 3.21 (br d, J= 9.2 Hz), and from the coupling constants of the 12β-H signal at δ 5.26 (dt, J= 2.9 and 2.3 Hz), respectively. The β–orientation of the furan ring, assumed from the biogenesis of this type of C-seco limonoids,[11] was also confirmed from the coupling of the 17α-H signal at δ 3.41 (br d, J= 9.5 Hz).

Nimbolinin D (**7**), $C_{38}H_{46}O_9$, showed the presence of hydroxyl (3450 cm^{-1}), ester (1735 cm^{-1}) and conjugated ester (1710 cm^{-1}) groups and olefinic double bond (1640 cm^{-1}) in the IR spectrum. The ^{1}H and ^{13}C NMR spectra showed the presence of an acetyl and a cinnamoyl group and an acetal carbon (δ 97.9), along with a furan ring. These NMR spectral data strongly suggested that **7** also had a nimbolinin-type structure such as **6**. The ^{1}H NMR spectrum of **7** is very similar to that of 12-*O*-methylvolkensin (**2**) being the first 12-*O*-methyl natural product in this type isolated from an another Chinese specimen of *M. toosendan*,[6] particularly with respect to the presence of a methoxy group at δ 3.02 (s, 12-OMe) and an isolated AB-type methylene due to the C-28/C-6 oxymethylene bridge at δ 3.61 (d, J= 7.6 Hz, H-28β) and 3.68 (br d, J= 7.6 Hz, H-28α), weakly coupled to 4β-Me (29) at δ 1.21. These data suggested that **7** differed from **2** only in the change of a tigloyl group in **2** to a cinnamoyl group in **7**. The presence of a 1α-cinnamoyloxy group was deduced from the significant upfield shift of 12α-OMe signal to δ 3.02 (s), in analogy with that (δ 3.06) in **2**, and from a W-type long range coupling between 1β and 3β-H at δ 4.81 (br t, J= 2.5 Hz) and 4.95 (br t, J= 2.6 Hz). Similar upfield shifts were also observed on 11α-H, 11β-H and 12β-

H at δ 1.60 (br dd, J= 14.0 and 2.2 Hz), 1.80 (ddd, J= 14.0, 10.4 and 2.4 Hz) and 4.61 (br t, J= 2.3 Hz). This effect can be accounted for by an anisotropic effect of the cinnamoyl group.[10,12] Assignment of the

Table 1.   NMR Spectral data of Nimbolinins (6-8).

no.	6		7		8	
	H	C	H	C	H	C
1	4.85 br t (2.8)	71.6 d	4.81 br t (2.5)	71.1 d	3.64 br t (2.7)	71.1
2	2.20 m	27.9 t	2.24 br t (2.5)	27.8 t	2.19 m	29.2
3	4.95 br t (2.8)	72.4 d	4.95 br t (2.6)	71.6 d	4.88 br t (2.7)	72.3
4		42.6 s		42.7 s		42.5
5	3.01 d (12.9)	40.2 d	2.93 d (12.8)	38.6 d	2.96 d (13.2)	39.1
6	4.19 dd (12.9, 2.6)	72.4 d	4.08 dd (12.8, 2.9)	73.5 d	4.15 dd (13.2, 2.7)	72.8
7	5.95 d (2.6)	75.3 d	4.40 d (2.9)	74.0 d	5.88 d (2.7)	75.6
8		45.4 s		46.1 s		45.5
9	3.21 br d (9.2)	35.5 d	3.24 d (10.4)	34.9 d	3.17 dd (6.3, 3.4)	36.5
10		40.4 s		40.7 s		41.3
11α	1.65 dd (16.6, 2.3)	31.6 t	1.60 br dd (14.0, 2.2)	31.6 t	1.80 m	30.8
β	1.93 m		1.80 ddd(14.0, 10.4, 2.4)		1.80 m	
12	5.26 br dt (2.9, 2.3)	91.4 d	4.61 br t (2.3)	97.9 d	4.78 t (2.0)	98.7
13		141.5 s		139.1 s		142.4
14		142.7 s		144.1 s		143.0
15	5.17 d (7.7)	78.0 d	5.03 br d (7.8)	77.7 d	4.90 br d (8.0)	77.7
16α	1.98 m	38.1 t	2.54 ddd (14.5, 9.0, 7.8)	37.9 t	1.91 m	37.7
β	1.36 br d (14.1)		1.58 br d (14.5)		1.42 br d (14.0)	
17	3.41 br d (9.5)	46.4 d	3.45 d (9.0)	46.9 d	3.20 br d (9.3)	46.8
18	1.83 s	16.2 q	1.76 s	16.2 q	1.82 br s	16.4
19	1.05 s	16.1 s	0.99 s	16.1 s	0.94 s	16.2
20		128.4 s		128.7 s		128.2
21	7.20 br s	142.8 d	7.27 br s	142.7 d	7.20 br d (1.5)	142.8
22	6.32 br d (1.4)	110.2 d	6.42 br s	110.5 d	6.34 br d (1.5)	110.4
23	7.28 br t (1.5)	138.9 d	7.30 br t (1.3)	139.0 d	7.27 t (1.5)	139.0
28α	3.41 br d (7.3)	77.6 t	3.68 br d (7.6)	78.0 t	3.47 br d (14.4)	78.1
β	3.50 d (7.3)		3.61 d (7.6)		3.45 d (14.4)	
29	1.20 s	19.2 q	1.21 s	19.6 q	1.14 s	19.0
30	1.53 s	20.7 q	1.37 s	20.8 q	1.48 s	21.1
OMe			3.02 s	54.2 q	3.37 s	54.4
CO*Me*	1.92 s	20.8 q	1.90 s	21.1 q	2.00 s	20.6
	2.24 s	21.8 q				
*CO*Me		169.7 s		170.3 s		170.7
		170.0 s				
COR	(benzoyl)		(cinnamoyl)		(benzoyl)	
1'		164.7 s		166.0 s		164.9
2'		130.4 s	6.51 d (16.1)	119.3 d		130.6
3'	8.17 dd (7.7, 1.3)	129.3 d	7.76 d (16.1)	144.1 d	8.11 dd (7.0, 1.5)	129.5
4'	7.43 t (7.7)	128.4 d		130.2 s	7.43 t (7.5)	128.4
5'	7.57 tt (7.5, 1.3)	133.0 d	7.40 m	127.9 d	7.55 tt (7.5, 1.5)	132.8
6'	7.43 t (7.7)	128.4 d	7.54 m	129.0 d	7.43 t (7.5)	128.4
7'	8.17 dd (7.7, 1.3)	129.3 d	7.40 m	128.7 d	8.11 dd (7.0, 1.5)	129.5
8'			7.54 m	129.0 d		
9'			7.40 m	127.9 d		

Chemical shift values are in ppm from TMS, and J values (in Hz) are presented in parentheses

relative configuration at the five chiral centers of the B and C rings was established from NOE experiments. NOE correlation from 15-H at δ 5.03 (br d, J= 7.8 Hz) to 12α-OMe and 9α–H at δ 3.24 (d, J= 10.4 Hz), suggested their same α-orientation.   On the other hand, a NOE between 7β–H at δ 4.40 (d, J= 2.9 Hz) and the vinylic 13-Me (18) at δ 1.76, showing homoallyl couplings with the 15 and 17-H signals, revealed that they were spatially close each other.   This type of 12-methoxy acetal compound is the second occurrence in a limonoid.

Nimbolinin D (**8**), $C_{36}H_{44}O_9$, showed similar IR and NMR spectra to **7** except for changing a cinnamoyl group in **7** to a benzoyl group in **8**.   The presence of the 7α–benzoyl and 3α-acetoxy groups was deduced from a similar downfield shift of 7β–H to δ 5.88 (d, J= 2.7 Hz) in **8** to δ 5.95 in **6** and from a NOE between 3β–H at δ 4.88 (br t, J= 2.7 Hz) and 4β-Me (29) at δ 1.14 , respectively.   A hydroxyl group should be present at 1α because a W-type long range coupling was observed between 1β-H at δ 3.64 (br t, J= 2.7 Hz) and 3β-H.   Thus, the 12-*O*-Me group was observed at the normal field of δ 3.37 in **8** different from δ 3.02 in **7**.   NOEs were also observed from 15-H at δ 4.90 (br d, J= 8.0 Hz) to 9-H at δ 3.17 (dd, J= 6.3 and 3.4 Hz) and 12-OMe.   The NOE correlation of 7β–H with the vinylic 13-Me (18) at δ 1.82 deduced the same B/C and C/D ring junction of **8** as **7**.

The antifeedant activity of the isolated compounds was tested against the third-instar larvae of *Spodoptera littoralis* (Boisduval) by a conventional leaf disk method.[13]   The most potent was toosendanin (**11**), which was active at 200 ppm, corresponding to the concentration of *ca.* 4 μg/cm^2.   Nimbolinins, (**6**)-(**9**), and trichilinins, (**4**), (**5**) and (**10**), showed weak activity at 1000 ppm.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ with 400 and 100 MHz, respectively, using TMS as internal standard on a JEOL FX-400 spectrometer.   IR (KBr) and UV (in MeOH) were recorded on JASCO FT/IR 5300 and Shimadzu UV-210A spectrophotometers.   Optical rotation was obtained at 22° C on a JASCO DIP-370S spectropolarimeter.

Plant material.   The fruits of *Melia toosendan* were collected in July 1995 at Guangzou, China.

Extraction and Isolation.   The fresh fruits (1.4 kg) were extracted with acetone (20 L) for 2 weeks at rt to give an extract (127 g).   The acetone extract (31 g) was divided into *n*-hexane-, CH_2Cl_2- and MeOH-soluble fractions.   The CH_2Cl_2 fraction (2.27 g) was flash chromatographed on silica gel with a MeOH-

CH$_2$Cl$_2$ solvent system. Each of the limonoid fraction eluting with 3% MeOH-CH$_2$Cl$_2$ was purified through HPLC using μ-Bondapac C$_{18}$ with 25-40% H$_2$O-MeOH as the solvent to give **4** (3 mg), **5** (2.5 mg), **10** (1.6 mg), **9** (2 mg), **6** (1.5 mg), **7** (1 mg), **8** (3.7 mg) and **11** (1 mg),

Nimbolinin A (6). An amorphous powder, C$_{37}$H$_{44}$O$_{10}$; HRFABMS m/z 671.2838 [M+Na]$^+$ (Δ +0.5 mmu); [α]$_D$ –35˚ (c 0.075, MeOH); UV 217 (ε 10000) and 228 nm (ε 13000); IR 3450, 1740-1710, 1660, 1600 and 1580 cm^{-1}.

Nimbolinin C (7). An amorphous powder, C$_{38}$H$_{46}$O$_9$; CIMS m/z 647 [M+1]$^+$; [α]$_D$ +30˚ (c 0.075, CH$_2$Cl$_2$); UV 222 (ε 15000) and 227 nm (ε 15000); IR 3450, 1735, 1710, 1640 cm^{-1}.

Nimbolinin D (8). An amorphous powder, C$_{36}$H$_{44}$O$_9$; CIMS m/z 621 [M+1]$^+$; [α]$_D$ –48˚ (c 0.015, MeOH); UV 208 (ε 12000) and 227 nm (ε 14000); IR 3470, 1726, 1650 and 1600 cm^{-1}.

Antifeedant activity. The antifeedant potential of the isolated limonoids was tested by a conventional leaf disk method using Chinese cabbage (*Brassica campestris* L. var. **chinensis**) against the third-instar larvae of *S. littoralis* (Boisduval). The test was assessed by presenting each test compound on leaf disks to larvae and by comparing the treated and untreated leaves eaten by the larvae. The feeding bioassay was terminated after the larvae had eaten approximately 50% of the control disk. To determine the minimum inhibitory concentration, this choice test was done at 100, 200, 300, 500 and 1000 ppm.

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