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RING C-SECO LIMONOIDS FROM *MELIA TOOSENDAN*

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Key Word Index—*Melia toosendan*; Meliaceae; antifeedants; 3-*O*-acetylohchinolal; ohchinolide C; nimbolidin F; salannin; acetyltrichilenone; 20,21,22,23-tetrahydro-23-oxotrichilenone; azadirone.

Abstract—Three different types of ring C-seco limonoids, 3-*O*-acetylohchinolal, ohchinolide C and nimbolidin F, were isolated as insect antifeedants from *Melia toosendan* along with three known limonoids, salannin, azadirone and acetyltrichilenone, and a known protolimonoid, 20, 21, 22, 23-tetrahydro-23-oxotrichilenone. Their structures were elucidated by spectroscopic studies and their antifeedant properties were examined with the larvae of *Spodoptera eridania*. © 1997 Elsevier Science Ltd

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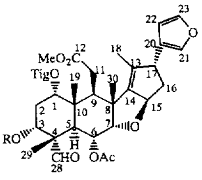
INTRODUCTION

In our continuous study of limonoids from *M. toosendan* [1], we isolated three new ring C-seco limonoids, 3-*O*-acetylohchinolal (**1**), ohchinolide C (**2**) and nimbolidin F (**3**), along with one C-seco limonoid, salannin (**4**) [2], two intact apo-euphol limonoids, azadirone (**5**) [3] and acetyltrichilenone (**6**) [4], and a protolimonoid, butanolide (**7**) [5], from the root bark. Compound **1** is the second limonoid of this type [6] and one of the biogenetic precursors to the ring-C seco-limonoids having C-6/C-28 and C-7/C-15 ether linkages, such as in salannin, and ohchinin (**11**) [6]. C-Seco limonoids are major ring-seco limonoids in *M. azedarach*, *M. azadirachta* and *M. toosendan* in the family Meliaceae and found only in these related species. The butanolide (**7**) was first isolated from *Melia* species. Insect antifeedant activity of the isolated compounds was tested against the larvae of the voracious pest insect *Spodoptera eridania* (Boisduval).

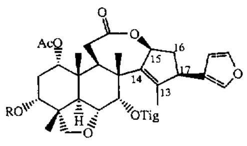
RESULTS AND DISCUSSION

An oily mixture, soluble in 50% hexane–diethyl ether, obtained from the ether extract of air-dried root bark was fractionated using silica flash chromatography and preparative TLC. HPLC purification of the limonoid fractions gave compounds **1**–**3** along with four known tetranortriterpenoids (**4**–**7**), as well as six limonoids reported previously [1].

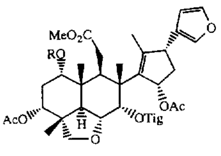
3-*O*-Acetylohchinolal (**1**), $C_{36}H_{46}O_{11}$, FAB-mass spectrum *m/z*: 655 $[M+1]^+$, $[\alpha]_D + 62°$ (*c* 0.14, MeOH), exhibited the presence of formyl (2957 and 1710 cm^{-1}), ester (1740 cm^{-1}) and conjugated ester (1710 cm^{-1}) groups in the IR spectrum. The ^{13}C NMR and ^1H NMR (at 27 and 45°) spectra indicated that **1**



1: R=Ac
8: R=H



2: R=COCH(CH$_3$)$_2$
9: R=Ac



3: R=COCH(CH$_3$)CH$_2$CH$_3$
10: R=Tig

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contained nine CH_3 (one methoxy), three CH_2, thirteen CH (one formyl and four olefinic) and eleven carbons (four ester carbonyl and four olefinic) not bonded to hydrogen. Limonoids possessing a formyl group have been rarely observed in nature and the NMR data of **1** suggested that the structure was similar to that of ohchinolal (**8**) [6], isolated from the fruits of *M. azedarach* from Japan.

The ^{1}H NMR spectrum of **1** was superimposable on that of **8** except for an addition of one acetyl group and some differences in the chemical shifts. Different chemical shifts of the 1-H and 9-H signals at δ 4.79 and 2.94 in **1** from δ 5.07 and 2.84 in **8** strongly suggested the tigloyloxy group to be substituted at C-3, but the fact that **1** has a 1α-tigloyloxy and 3α-acetoxy structure was elucidated by a NOE observation between 9-H and the 3′-Me signal of the tiglate. Other NOEs between the 5-H and 28-formyl proton signals, the 9-H and 15-H signals and the 8-Me and 6-H and 7-H signals showed the stereochemistry of **1** to be the same as that of **8**.

Compound **2**, $C_{37}H_{48}O_{10}$, $[\alpha]_D - 23°$ (c 0.11, MeOH), named ohchinolide C, showed the presence of ester (1740 cm^{-1}) and conjugated ester (1710 cm^{-1}) groups and olefinic double bonds (1640 and 1620 cm^{-1}) in the IR spectrum. The ^{1}H NMR data indicated that **2** contained each one of acetyl, 2-methylpropanoyl, tigloyl and olefinic methyl groups along with a furan moiety. The ^{1}H NMR and ^{13}C NMR spectra of **2** were superimposable on those of ohchinolide B (**9**) isolated from *M. azedarach* from Japan [7] and the former Yugoslavia [8] except that one acetyl group in **9** was

Table 1. ^{1}H NMR data for compounds **1**, **2** and **3** (400 MHz, $CDCl_3$)

H	**1**	**2**	**3**
1	4.79 *t* (2.6)	4.83 *t* (2.8)	4.56 *dd* (2.6, 2.0)
2α	2.11 *dt* (16.5, 2.6)	2.22 *dt* (17.6, 2.8)	2.26 *m*
β	2.27 *dt* (16.5, 2.6)	2.27 *dt* (17.6, 2.8)	2.27 *m*
3	4.82 *t* (2.6)	4.97 *t* (2.8)	4.94 *dd* (3.5, 2.0)
5	3.71 *d* (12.5)	2.71 *d* (12.6)	2.75 *d* (12.6)
6	5.26 *dd* (12.5, 2.6)	4.08 *dd* (12.6, 2.8)	4.13 *dd* (12.6, 3.0)
7	4.04 *d* (2.6)	5.71 *d* (2.8)	5.56 *d* (3.0)
9	2.94 *dd* (7.1, 4.3)	3.17 *dd* (12.3, 4.5)	3.54 *t* (6.3)
11α	2.24 *dd* (15.4, 4.3)	2.67 *dd* (18.6, 4.5)	2.20–2.30
β	2.29 *dd* (15.4, 7.1)	2.77 *dd* (18.6, 12.3)	2.20–2.30
15	5.54 *t* (*br*) (6.6)	5.50 *d* (7.2)	5.73 *d* (*br*) (6.8)
16α	2.27 *dd* (*br*) (12.3, 6.6)	2.36 *ddd* (15.0, 9.5, 7.2)	2.38 *ddd* (14.9, 8.8, 6.8)
β	2.09 *ddd* (12.3, 8.8, 6.6)	1.97 *d* (15.0)	1.57 *d* (14.9)
17	3.65 *d* (*br*) (8.8)	3.43 *d* (9.5)	3.44 *d* (*br*) (8.8)
18(Me)	1.66 *d* (1.3)	1.80 *s* (*br*)	1.95 *s* (*br*)
19(Me)	1.10 *s*	1.07 *s*	1.14 *s*
21	7.27 *m*	7.26 *m*	7.18 *m*
22	6.31 *s* (*br*)	6.31 *dd* (*br*) (1.1, 0.7)	6.19 *d* (*br*) (0.8)
23	7.34 *t* (*br*) (1.5)	7.31 *t* (*br*) (1.4)	7.23 *m*
28α	9.64 *s*	3.48 *d* (*br*) (7.4)	3.30 *d* (*br*) (7.2)
β		3.55 *d* (7.4)	3.49 *d* (7.2)
29(Me)	1.09 *s*	1.20 *s*	1.21 *s*
30(Me)	1.41 *s*	1.45 *s*	1.25 *s*
CO_2Me	3.28 *s*	—	3.55 *s*
1-Ac	—	1.92 *s*	—
3-Ac	1.97 *s*	—	2.13 *s*
6-Ac	1.84 *s*	—	—
15-Ac	—	—	1.99 *s*
2-Methylpropanoyl and 2-Methylbutanoyl			
2′	—	2.51 *hept* (7.1)	2.15 *m*
2′-Me	—	1.21 *d* (7.1), 1.24 *d* (7.1)	1.24 *d* (6.6)
3′	—	—	1.45 *m*, 1.88 *m*
3′-Me	—	—	0.95 *t* (7.3)
Tig			
2′-Me	1.97 *s* (*br*)	1.93 *t* (*br*) (1.1)	1.79 *s* (*br*)
3′	6.99 *qq* (7.3, 1.5)	7.05 *qq* (7.1, 1.4)	6.78 *dq* (7.0, 1.3)
3′-Me	1.83 *dq* (7.3, 1.0)	1.84 *dq* (7.0, 1.1)	1.68 *d* (*br*) (7.0)

changed to a 2-methylpropanoyl group. The presence of a 2-methylpropanoyl group at C-3α of **2** was demonstrated by NOEs between the 2-methylpropanoyl dimethyl signals at δ 1.21 and 1.24 and the 5-H and 28α-H signals at δ 2.71 and 3.48. The *S*-configuration at C-15 was also elucidated from a NOE enhancement of the 7β-H signal at δ 5.71 by irradiation of the olefinic 13-Me signal at δ 1.80.

The structure of nimbolidin F (**3**), $C_{41}H_{56}O_{12}$, $[\alpha]_D +4°$ (*c* 0.09, MeOH), was elucidated from the IR (1736, 1710, 1640 and 1620 cm^{-1}) and ^1H NMR data. Their spectra were similar to those of nimbolidins from the same plant [3], especially to that of nimbolidin D (**10**) except for the change of the 1α-tigloyloxy group in **10** to a 2-methylbutanoyl group in **3**, accompanying a high field shift of the 1β-H signal at δ 4.76 in **10** to δ 4.56 and a low field shift of the 12-CO_2Me signal at δ 3.50 in **10** to δ 3.55.

The insect antifeedant activity of the isolated compounds **1**–**7**, were tested against the larvae of *S. eridania* (Boisduval) by a conventional leaf disk method [9]. Nimbolidin F (**3**) showed the activity at 500 ppm, corresponding to the concentration 10 μg ml^{-1}, and acetylohchinolal (**1**), ohchinolide C (**2**) and salannin (**4**) were active at 1000 ppm. On the other hand, intact apo-euphol limonoid, **5** and **6**, and the protolimonoid **7** were not active at 1000 ppm.

EXPERIMENTAL

^1H and ^{13}C NMR: with 400 and 100 MHz in $CDCl_3$, (Tables 1 and 2). $[\alpha]_D$, CD and UV: in MeOH. IR: with KBr.

Plant material. The root bark was collected in December 1992 at Xiangtan, China. The plant material was identified by Dr Wen Xue and a voucher specimen is deposited in the herbarium at Department of Biology, Normal University of Xiangta, P.R. China.

Extraction and isolation. The air-dried root bark (1.5 kg) was defatted with hexane (20 l.) and then extracted with Et_2O (20 l.) to yield 12.8 g of an ether extract, which was dissolved in 50 ml of Et_2O and then added to the same vol. of hexane to give 9.1 g of a soluble part. It was flash chromatographed on silica gel with a hexane–Et_2O solvent system. Each of the limonoid frs eluted with Et_2O was purified by prep. TLC using C_6H_6–MeOH (3:1) to give three limonoid frs. Each fr was further sepd and purified through HPLC using μ-Bondapac C_{18} with 20–45% H_2O–MeOH as the solvent to give **1** (6.7 mg), **2** (1.1 mg), **3** (0.9 mg), **4** (4.2 mg), **5** (5.0 mg), **6** (18.0 mg) and **7** (2.0 mg).

3-O-acetylohchinolal (**1**). An amorphous powder, $C_{36}H_{46}O_{11}$: FAB-MS *m/z*: 655 $[M+H]^+$, $[\alpha]_D +62°$ (*c* 0.14); UV λ_{max} nm (ε): 222.5 (9800), 275 (1400); IR ν_{max} cm^{-1}: 2957, 1740, 1710, 1638 and 1618; CD: $\Delta\varepsilon_{213} +17.6$, $\Delta\varepsilon_{220} +15.3$.

Table 2. ^{13}C NMR data for compounds **1** and **2** (100 MHz, $CDCl_3$)

C	**1**	**2**
1	70.8 *d*	71.5 *d*
2	30.5 *t*	27.8 *t*
3	75.1 *d*	71.8 *d*
4	47.1 *s*	42.4 *s*
5	39.5 *d*	40.6 *d*
6	69.1 *d*	70.8 *d*
7	85.3 *d*	73.9 *d*
8	47.5 *s*	45.0 *s*
9	35.9 *d*	37.3 *d*
10	41.9 *s*	40.8 *s*
11	25.0 *t*	32.7 *t*
12	172.5 *s*	171.2 *s*
13	135.7 *s*	138.6 *s*
14	146.1 *s*	146.8 *s*
15	87.6 *s*	85.4 *d*
16	41.2 *t*	37.6 *t*
17	49.6 *d*	47.0 *d*
18	17.0 *q*	16.0 *q*
19	16.7 *q*	15.5 *q*
20	126.9 *s*	126.5 *s*
21	143.0 *d*	139.2 *d*
22	110.6 *d*	110.0 *d*
23	138.9 *d*	143.5 *d*
28	204.3 *d*	78.0 *t*
29	14.4 *q*	18.9 *q*
30	13.0 *q*	20.5 *q*
Ac	170.1 *s*	170.3 *s*
	169.1 *s*	—
	20.9 *q*	20.8 *q*
	20.5 *q*	—
CO_2Me	51.6 *q*	—
Tig		
1′	166.6 *s*	165.2 *s*
2′	128.9 *s*	129.0 *s*
2′-Me	12.0 *q*	12.2 *q*
3′	137.7 *d*	137.6 *d*
3′-Me	14.1 *q*	14.4 *q*
2-methylpropanoyl		
1′	—	174.8 *s*
2′	—	34.7 *d*
2′-Me	—	19.5 *q*
3′	—	19.5 *q*

Ohchinolide C (**2**). An amorphous powder, $C_{37}H_{48}O_{10}$: HRFAB-MS *m/z*: 653.3347 $[M+1]^+$ (Δ+2.1 mmu); $[\alpha]_D -23°$ (*c* 0.11); UV λ_{max} nm (ε): 208.6 (11 600); IR ν_{max} cm^{-1}: 1740, 1710, 1640 and 1620.

Nimbolidin F (**3**). An amorphous powder, $C_{41}H_{56}O_{12}$: CI-MS *m/z*: 741 $[M+H]^+$; $[\alpha]_D +4°$ (*c* 0.09); UV λ_{max} nm (ε): 216 (11 800); IR ν_{max} cm^{-1}: 1736, 1710, 1640 and 1620.

Antifeedant activity. The antifeedant potential of the isolated compounds was tested by a conventional leaf disc method using Chinese cabbage (*Brassica campestris* L. var *chinensis*) [9] against the third-instar larvae of *S. eridania*. From the choice test at 300, 400, 500 and 1000 ppm, the minimum inhibitory concn of each limonoid was determined: nimbolidin F (**3**): 500 ppm; acetylohchinolal (**1**), ohchinolide C (**2**) and salannin (**4**): 1000 ppm; azadiron (**5**), acetyltrichilenone (**6**) and the butanolide (**7**): not active.

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