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# NIMBOLIDINS C–E, LIMONOID ANTIFEEDANTS FROM *MELIA TOOSENDAN*

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**Key Word Index**—*Melia toosendan*; Meliaceae; Nimbolidins C, D and E; salannin; limonoid.

**Abstract**—Three new ring C-seco-limonoids, nimbolidins C–E, were isolated from the root bark of *Melia toosendan* along with two known seco-limonoids, nimbolidin B and salannin. These compounds have properties as insect feeding inhibitors. Their structures were elucidated by spectroscopic means.

## INTRODUCTION

Limonoids from *Melia* species are attracting considerable interest, because of a variety of structures and insect feeding inhibitor properties. We have isolated several types of limonoids, meliacarpinins [1, 2], trichilins [3–5] and azedarachins [6, 7], as insect 'antifeedants' from Okinawan and Chinese *M. azedarach* L. and a related plant *M. toosendan*. In the continuous study of antifeedants from *M. toosendan*, we isolated three new ring C-seco-limonoids, named nimbolidins C, D and E, along with two known seco-limonoids, nimbolidin B [8] and salannin [9], from the root bark. 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 disc method [10] against the larvae of the Japanese pest insect *Spodoptera eridania* (Boisduval).

## RESULTS AND DISCUSSION

Three new limonoids, nimbolidins C (**1**), D (**2**) and E (**3**), were isolated along with two known limonoids, nimbolidin B (**4**) and salannin (**5**), from the hexane extract of the dried root bark by flash chromatography and preparative TLC, followed by careful HPLC using a reversed-phase column.

Nimbolidin C (**1**), $[\alpha]_D^{23}$ + 14° (MeOH, *c* 0.3) showed the presence of estercarbonyls (1735 $cm^{-1}$) and double bonds (1640 and 1620 $cm^{-1}$) in the infrared spectrum. The $^{13}C$ NMR and mass spectral data revealed the molecular formula to be $C_{37}H_{50}O_{12}$ (13 unsaturations). Extensive NMR studies including COSY, DEPT spectra and NOE experiments, allowed us to derive the structure **1**. Some pertinent points related to the structural study were as follows. The $^{13}C$ NMR and $^1H$ NMR spectra (at 27° and 45°) indicated that **1** contained 10 Me, four $CH_2$, 12 CH, and 11 C (five carbonyls) not bonded to hydrogen, including one tetra-substituted double bond, and no proton due resulting from a hydroxyl group. The $^1H$ NMR spectrum revealed the presence of a typical 2-methylpropanoyl and three acetoxyl substituents and a 3-furyl moiety. The additional presence of carbomethoxy and olefinic methyl groups at $\delta$3.57 and 2.10 and a characteristic 28-methylene group, $\delta$3.46 and 3.49 (each *d*, *J* = 7.6 Hz), forming an ether linkage with C-6 like **5**, strongly suggested **1** to be a salannin-type ring C-seco-limonoid except for the following details.

(1) The signals at $\delta$4.18 and 5.12 due to the protons attached to C-7 and C-15 forming an ether linkage in **5** changed to $\delta$ 5.42 and 5.74 and are assigned to the protons associated with acyloxyl groups in **1**. This observation suggested **1** to be a limonoid like nimbolidin B (**4**), isolated from *M. azedarach* from the former Yugoslavia [8]. Moreover, the NMR data of **1** were superimposable on those of **4** except for the change of an acyl substituent at C-7 to 2-methylpropanoyl assigned by the NOEs between one 2′-Me signal at $\delta$1.05 and the 13-Me signal at $\delta$2.10 and the other 2′-Me signal at $\delta$1.21 and the 15-H signal at $\delta$5.74. The other acyloxyl groups were then acetoxyls and the presence of one at C-15$\beta$ was confirmed from the coupling (*d*, *J* = 7.0 Hz) of the 15$\alpha$-H signal at $\delta$ 5.74 with the 16$\alpha$-H signal (*ddd*, *J* = 15.0, 9.3 and 7.0 Hz) at $\delta$ 2.43, coupled to 16$\beta$-H (*d*, *J* = 15.0 Hz) and 17$\alpha$-H (*br d*, *J* = 9.3 Hz) at $\delta$1.63 and 3.50, respectively. These couplings clarified that the 15-H and 17-H were situated on the same side of the D ring. As the stereochemistry at C-17 in the limonoid is evident from the biogenesis, the configuration at C-15 was determined

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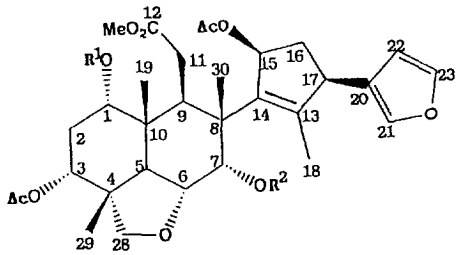
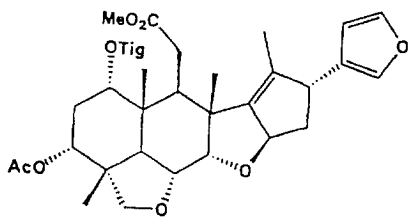


| | $R^1$ | $R^2$ |
|---|---|---|
| **1** | Ac | $COCH(Me)_2$ |
| **2** | Tig | Tig |
| **3** | Tig | $COCH(Me)_2$ |
| **4** | Ac | Tig |



**5**

to be *S* and no NOE observation between the isobutanoyl and 15-OAc groups showed a preferential conformation of the ring D as shown in the structure.

(2) The substitution pattern around the A ring, namely, that **1** has 1α- and 3α-acetoxyl groups, was the same as that in **4** and different from the 1α-tigloyloxyl and 3α-acetoxyl groups in **5**. Their α-configurations were assigned from a long range *W*-bond coupling between the equatorial 1β- and 3β-H at δ 4.43 and 4.91.

Further stereochemistry in **1** was confirmed from a long-range coupling of the 9α-H at δ3.65 with the 30-Me at δ1.21 and NOEs between the 30-Me signal and the 7β-H signal at δ5.42 and the 29-Me signal at δ1.20 and the 3β- and 6β-H signals at δ4.91 and 4.10.

The structure of the second limonoid, nimbolidin D; $C_{41}H_{54}O_{12}$, $[\alpha]_D^{23}$ − 55° (*c* 0.6), was readily elucidated to be **2** from the similarity of the $^1$H NMR spectrum to that of **1**, except for the presence of two acetoxyl groups and a tigloyloxyl group in **2** instead of three acetoxyls and one 2-methylpropanoyl in **1**. In the spectrum of **2**, two signals due to 1β and 7β-H showed downfield shifts to δ4.76 and 5.58 from δ4.43 and 5.42 in **1** suggest strongly the presence of tigloyloxyl groups at C-1 and C-7 in **2**, in consideration of the data from related ring C cleaved limonoids [8, 11]. Conversely, slight upfield shifts of the 9-H (Δδ0.09 ppm) and 12-$CO_2$Me (Δδ0.07 ppm) signals were also observed, supporting the presence of a 1α-tigloyloxyl group [9].

The $^1$H NMR spectrum of the third limonoid, nimbolidin E (**3**), $[\alpha]_D^{22}$ + 4° (*c* 0.4), with a molecular formula $C_{40}H_{54}O_{12}$, was also superimposable on that of **1**, except that the acetoxyl group at C-1 in **1** changed to tigloyloxyl in **3**, which was deduced by a low shift to δ4.67 of the 1β-H signal. Upfield shifts of the 9-H (Δδ0.11 ppm) and 12-$CO_2$Me (Δδ0.04 ppm) signals through the effect of the tigloyl group were also observed. From these $^1$H NMR data and the $^{13}$C NMR spectrum, the structure **3** was elucidated.

The insect antifeedant activity of the isolated compounds **1**–**5**, were tested by a conventional leaf disc method [10] against the larvae of *S. eridania*. Nimbolidins **1**–**4** showed activity at 500 ppm, corresponding to the concentration of 10 μg cm$^{-2}$, which is weaker than that of trichilins and azedarachins (200–400 ppm) [12] from the same plant. However, salannin (**5**) was active at 1000 ppm.

## EXPERIMENTAL

$^1$H NMR and $^{13}$C NMR: 400 and 100 MHz in $CDCl_3$, Tables 1 and 2. $[\alpha]_D$, UV and CD: in MeOH. IR: KBr. Bioassay of the antifeedant was done by the leaf-disc method against the third instar larvae of *S. eridania*.

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

*Extraction and isolation.* The air-dried root bark (1.5 kg) was extracted with hexane (20 l). at 15° for 2 weeks, to yield 9.3 g of an extract, which 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 $Et_2O$ 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** (6.6 mg), **2** (4.4 mg), **3** (5.0 mg), **4** (0.9 mg) and **5** (4.2 mg).

*Nimbolidin C* (**1**). Amorphous powder, $C_{37}H_{50}O_{12}$, $[\alpha]_D^{23}$ + 14° (*c* 0.3); positive FAB-MS *m/z*: 709 $[M + Na]^+$, negative FAB-MS *m/z*: 685 $[M - H]^-$, HRFAB-MS *m/z*: 627.3179 $[M + H - AcOH]^+$ (Δ + 1.0 mmu); UV $\lambda_{max}$ nm (ε): 217 (15000); IR $\nu_{max}$ cm$^{-1}$: 1735, 1640, 1620.

*Nimbolidin D* (**2**). Amorphous powder, $C_{41}H_{54}O_{12}$, $[\alpha]_D^{23}$ − 55° (*c* 0.6); positive FAB-MS *m/z*: 761 $[M + Na]^+$, negative FAB-MS *m/z*: 737 $[M - H]^-$; UV $\lambda_{max}$ nm (ε): 217 (14300); IR $\nu_{max}$ cm$^{-1}$: 1734, 1710, 1640, 1620.

*Nimbolidin E* (**3**). Amorphous powder, $C_{40}H_{54}O_{12}$, $[\alpha]_D^{22}$ + 4° (*c* 0.4); positive FAB-MS *m/z*: 749 $[M + Na]^+$, negative FAB-MS *m/z*: 725 $[M - H]^-$; UV $\lambda_{max}$ nm (ε): 217 (15800); IR $\nu_{max}$ cm$^{-1}$: 1736, 1710, 1640, 1620.

*Nimbolidin B* (**4**). Amorphous powder, $C_{38}H_{50}O_{12}$, $[\alpha]_D^{23}$ − 7° (*c* 0.15); positive FAB-MS *m/z*: 721 [M +

Table 1. $^1$H NMR data for nimbolidins **1**–**4** and salannin **5** (400 MHz, $CDCl_3$)

| H | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 4.43 *t* (2.6) | 4.76 *t* (2.6) | 4.67 *t* (2.6) | 4.47 *t* (2.6) | 4.79 *t* (2.9) |
| 2α | 2.24 *dt* (16.5, 2.7) | 2.26 *dt* (16.6, 2.9) | 2.24 *dt* (16.6, 2.7) | 2.27 *dt* (16.6, 2.8) | 2.20 *m* |
| 2β | 2.17 *dt* (16.5, 3.0) | 2.12 *dt* (16.5, 2.4) | 2.14 *dt* (16.6, 2.8) | 2.19 *dt* (16.6, 3.1) | 2.20 *m* |
| 3 | 4.91 *t* (2.7) | 4.96 *t* (3.0) | 4.94 *t* (2.8) | 4.93 *t* (2.8) | 4.96 *t* (2.9) |
| 5 | 2.71 *d* (12.8) | 2.78 *d* (12.5) | 2.72 *d* (12.5) | 2.83 *d* (12.5) | 2.82 *d* (12.6) |
| 6 | 4.10 *dd* (12.7, 2.8) | 4.15 *dd* (12.5, 3.3) | 4.13 *dd* (12.8, 3.3) | 4.14 *dd* (12.6, 2.7) | 3.99 *dd* (12.6, 3.2) |
| 7 | 5.42 *d* (2.9) | 5.58 *d* (3.3) | 5.44 *d* (3.3) | 5.57 *d* (2.9) | 4.18 *d* (3.3) |
| 9 | 3.65 *dd* (8.6, 4.3) | 3.54 *t* (6.7) | 3.56 *dd* (8.6, 5.6) | 3.70 *dd* (9.0, 5.1) | 2.75 *dd* (4.0, 8.1) |
| 11α | 2.23 *dd* (13.5, 4.4) | 2.21 *dd* (14.0, 6.4) | 2.22 *dd* (13.9, 5.7) | 2.25 *dd* (13.9, 5.2) | 2.20 *dd* (14.6, 4.4) |
| β | 2.32 *dd* (13.6, 9.2) | 2.27 *dd* (14.0, 6.5) | 2.31 *dd* (13.9, 8.5) | 2.32 *dd* (13.9, 5.2) | 2.31 *dd* (14.6, 8.1) |
| 15α | 5.74 *d* (*br*) (7.0) | 5.72 *d* (*br*) (6.6) | 5.73 *d* (*br*) (7.0) | 5.75 *d* (*br*) (7.3) | 5.44 *t* (*br*) (6.9) |
| 16α | 2.43 *ddd* (15.0, 9.3, 7.0) | 2.38 *ddd* (14.9, 6.6, 6.4) | 2.39 *ddd* (15.0, 9.2, 7.0) | 2.43 *ddd* (15.1, 9.1, 7.2) | 2.13 *ddd* (11.9, 8.8, 6.6) |
| β | 1.63 *d* (15.0) | 1.57 *d* (14.9) | 1.61 *d* (15.0) | 1.61 *d* (15.0) | 2.24 *dd* (11.9, 6.6) |
| 17 | 3.50 *d* (*br*) (9.3) | 3.38 *d* (*br*) (9.2) | 3.45 *d* (*br*) (9.2) | 3.46 *d* (*br*) (9.1) | 3.64 *d* (*br*) (8.8) |
| 18(Me) | 2.10 *s* (*br*) | 1.83 *s* (*br*) | 1.95 *s* (*br*) | 1.98 *s* (*br*) | 1.67 *d* (1.1) |
| 19 | 1.14 *s* | 1.13 *s* | 1.15 *s* | 1.15 *s* | 0.98 *s* |
| 21 | 7.26 *s* (*br*) | 7.16 *s* (*br*) | 7.24 *s* (*br*) | 7.19 *s* (*br*) | 7.26 *s* (*br*) |
| 22 | 6.23 *d* (0.7) | 6.19 *d* (0.7) | 6.21 *d* (0.7) | 6.21 *s* (*br*) | 6.30 *s* (*br*) |
| 23 | 7.30 *t* (1.5) | 7.23 *t* (1.5) | 7.30 *t* (1.5) | 7.25 *t* (*br*) (1.5) | 7.33 *t* (1.4) |
| 28α | 3.46 *d* (*br*) (7.6) | 3.42 *d* (*br*) (7.3) | 3.50 *d* (*br*) (6.6) | 3.42 *d* (*br*) (7.7) | 3.69 *d* (*br*) (7.6) |
| β | 3.49 *d* (7.6) | 3.50 *d* (7.3) | 3.47 *d* (6.7) | 3.49 *d* (7.7) | 3.58 *d* (7.4) |
| 29(Me) | 1.20 *s* | 1.21 *s* | 1.22 *s* | 1.21 *s* | 1.21 *s* |
| 30(Me) | 1.21 *s* | 1.24 *s* | 1.23 *s* | 1.24 *s* | 1.30 *s* |
| $CO_2$Me | 3.57 *s* | 3.50 *s* | 3.53 *s* | 3.58 *s* | 3.24 *s* |
| 1-Ac | 2.05 *s* | — | — | 2.08 *s* | 1.94 *s* |
| 3-Ac | 2.13 *s* | 2.12 *s* | 2.12 *s* | 2.15 *s* | — |
| 15-Ac | 1.93 *s* | 1.93 *s* | 1.86 *s* | 1.99 *s* | — |
| 7-iso Pro | | | | | |
| 2-H | 2.11 *quint* (6.6) | | 2.12 *quint* (6.7) | | |
| 2-Me | 1.05 *d* (6.6) | | 1.06 *d* (6.7) | | |
| 3(Me) | 1.21 *d* (6.6) | | 1.24 *d* (6.7) | | |
| 1-Tig | | | | | |
| 2-Me | | 1.89 *s* (*br*) | 1.88 *s* (*br*) | | 1.77 *s* (*br*) |
| 3-H | | 6.93 *qq* (6.9, 1.4) | 6.95 *qq* (7.0, 1.4) | | 6.96 *qq* (7.0, 1.3) |
| 3-Me | | 1.75 *d* (*br*) (7.0) | 1.74 *d* (*br*) (7.1) | | 1.81 *dq* (7.0, 1.1) |
| 7-Tig | | | | | |
| 2-Me | | 1.80 *s* (*br*) | | 1.81 *s* (*br*) | |
| 3-H | | 6.77 *qq* (6.9, 1.2) | | 6.80 *qq* (7.0, 1.3) | |
| 3-Me | | 1.72 *d* (*br*) (7.0) | | 1.70 *d* (*br*) (7.0) | |

Table 2. $^{13}C$ NMR data for nimbolidins **1**–**4** (100 MHz, $CDCl_3$)

| C | 1 | 2 | 3 | 4* |
|---|---|---|---|---|
| 1 | 71.5 *d* | 71.5 *d* | 71.9 *d* | 71.7 *d* |
| 2 | 27.2 *t* | 27.9 *t* | 27.7 *t* | 27.3 *t* |
| 3 | 72.8 *d* | 71.9 *d* | 72.0 *d* | 72.0 *d* |
| 4 | 42.6 *s* | 42.7 *s* | 42.7 *s* | 42.7 *s* |
| 5 | 40.0 *d* | 40.9 *d* | 40.5 *d* | 40.3 *d* |
| 6 | 71.8 *d* | 71.8 *d* | 72.0 *d* | 72.9 *d* |
| 7 | 75.2 *d* | 75.6 *d* | 75.3 *d* | 75.6 *d* |
| 8 | 47.4 *s* | 47.0 *s* | 47.3 *s* | 47.5 *s* |
| 9 | 38.7 *d* | 39.2 *d* | 38.9 *d* | 38.8 *d* |
| 10 | 40.7 *s* | 40.8 *s* | 40.9 *s* | 40.9 *s* |
| 11 | 32.0 *t* | 32.0 *t* | 32.0 *t* | 32.1 *t* |
| 12 | 173.6 *s* | 173.1 *s* | 173.2 *s* | 173.6 *s* |
| 13 | 133.5 *s* | 133.2 *s* | 133.5 *s* | 133.4 *s* |
| 14 | 148.5 *s* | 148.9 *s* | 148.7 *s* | 148.6 *s* |
| 15 | 82.0 *d* | 82.4 *d* | 82.2 *d* | 82.2 *d* |
| 16 | 36.7 *t* | 36.8 *t* | 36.7 *t* | 37.1 *t* |
| 17 | 47.5 *d* | 47.5 *d* | 47.4 *d* | 47.7 *d* |
| 18 | 16.9 *q* | 17.7 *q* | 17.0 *q* | 17.5 *q* |
| 19 | 16.0 *q* | 16.4 *q* | 16.4 *q* | 16.0 *q* |
| 20 | 127.8 *s* | 128.0 *s* | 127.9 *s* | 128.0 *s* |
| 21 | 139.5 *d* | 139.3 *d* | 139.5 *d* | 139.5 *d* |
| 22 | 109.8 *d* | 110.1 *d* | 109.9 *d* | 110.2 *d* |
| 23 | 143.2 *d* | 142.9 *d* | 143.2 *d* | 142.9 *d* |
| 28 | 77.8 *t* | 78.0 *t* | 77.8 *t* | 78.1 *t* |
| 29 | 20.0 *q* | 19.7 *q* | 20.1 *q* | 19.8 *q* |
| 30 | 20.1 *q* | 20.2 *q* | 20.2 *q* | 20.2 *q* |
| $CO_2$Me | 52.0 *q* | 51.7 *q* | 51.9 *q* | 52.08 *q* |
| OCOMe | 20.7 *q*, 21.3 *q* | 20.9 *q* | 20.7 *q* | 20.98 *q*, 21.3 *q* |
| | 21.6 *q* | 21.6 *q* | 21.6 *q* | 21.6 *q* |
| OCOMe | 169.7 *s* | 169.8 *s* | 169.9 *s* | 169.6 *s*, 169.9 *s* |
| | 170.0 *s* (×2) | 171.1 *s* | 171.0 *s* | 171.0 *s* |
| iso-Pro | | | | |
| 1′ | 174.7 *s* | | 174.9 *s* | |
| 2′ | 33.5 *d* | | 33.6 *d* | |
| 3′ | 17.6 *q*, 19.9 *q* | | 17.6 *q*, 19.8 *q* | |
| Tig | | | | |
| 1′ | | 166.6 *s*, 166.8 *s* | 166.5 *s* | 166.2 *s* |
| 2′ | | 129.4 *s*, 129.4 *s* | 129.4 *s* | 129.4 *s* |
| 2′-Me | | 12.1 *q*, 12.4 *q* | 12.0 *q* | 12.4 *q* |
| 3′ | | 135.9 *d*, 136.2 *d* | 136.4 *d* | 136.3 *d* |
| 3′-Me | | 14.2 *q*, 14.5 *q* | 14.1 *q* | 14.4 *q* |

*Ref. [8].

Na]$^+$, negative FAB-MS *m/z*: 697 $[M-H]^-$; UV $\lambda_{max}$ nm (ε): 219 (9800).

*Salannin* (**5**). Amorphous powder, $C_{34}H_{44}O_9$, $[\alpha]_D^{24}$ + 134° (*c* 0.6); CD nm: $\Delta\varepsilon_{216}$ + 17.

*Antifeedant activity*. The antifeedant potential of the isolated compounds was tested by placing them on 10 leaf disks (five treated with sample and five untreated disks as control) of a Chinese cabbage to the third instar larvae of *S. eridania* and visually comparing the treated and untreated leaves eaten by the larvae.

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