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Three New Antifeeding Meliacarpinins from Chinese *Melia Azedarach* Linn

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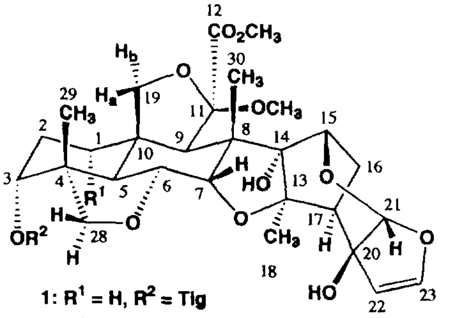
Abstract: Three new limonoid antifeedants, meliacarpinins 1-3, were isolated along with fourteen known limonoids from the root bark of Chinese *Melia azedarach* Linn (Meliaceae). Their structures and antifeedant activity were elucidated.

Meliaceae plants are a rich source of limonoids and the neem tree ***Melia azadirachta indica*** Juss and a related tree ***Melia azedarach*** L. are attracting considerable interest, particularly because of their insect antifeedant properties. The most potent constituent is azadirachtin,[1] a limonoid which exerts strong physiological and phagorepellent activities.[2] Several azadirachtin related compounds has been isolated[3] and their structure-activity relationship has been also investigated well.[4] ***M. azedarach*** is a native of Persia, India and China, but naturalized in a number of continents including Africa, Australia and Americas. Thus, the constituents of the tree have been studied in many regions to give degradated limonoids,[5] azadirachtin[6] and a related melianolone,[7] trichilin-type 19/29 bridged acyl acetals,[8] toosendanin[9] and a related compound,[10] salannin-type seco-limonoids,[11] and so on.

Recently, we isolated a new meliacarpinin **4** as an insect antifeedant from Okinawan ***M. azedarach***.[12] In the continuous study of limonoid antifeedants from the Chinese plant collected at Guangzhou, we have isolated three new meliacarpinins, named meliacarpinins B-D (**1-3**), along with **4**, seven trichilins, **5-11**,[13] four azedarachins, **12-14**,[14] and nimbolinin B (**16**)[11b] and salannin (**17**).[15] Their antifeeding activity was tested by a conventional leaf disk method against the Japanese voracious pest insects ***Spodoptera exigua*** Hübner and *S.* ***eridania*** (Boisduval). In this paper, we wish to report the structures of these meliacarpinins and the antifeedant activity of the isolated limonoids.



1: $R^1 = H, R^2 = Tig$
2: $R^1 = OAc, R^2 = Tig$
3: $R^1 = OTig, R^2 = Ac$
4: $R^1 = OCin, R^2 = Ac$
18: $R^1 = OTig, R^2 = Ac, 4\beta\text{-}CO_2CH_3$

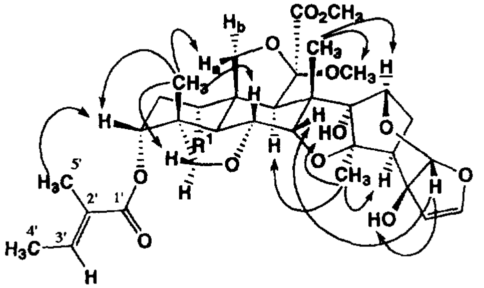
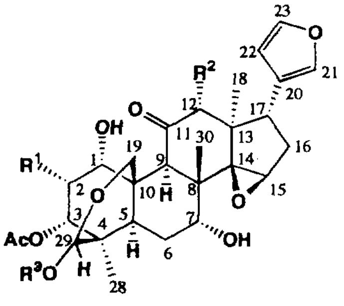


Fig. 1. Selective NOE connectivities for 1

RESULTS AND DISCUSSION

The ether extract of the root bark contained a variety of limonoids which were detected by the characteristic colour with Ehrlich's reagent on TLC. The limonoid antifeedants from *M. azedarach* were very sensitive to a trace of acid and gradually decomposed on a silica column. It was, therefore, necessary to use flash chromatography and HPLC separation techniques, and the isolation of the various congeners, which was monitored by antifeedant assay, was a tedious process requiring careful combined use of normal- and reversed-phase HPLC.

The ether extract of the root bark was divided into soluble and insoluble parts into ether containing 50% hexane. The insoluble part gave **1**–**3** along with 14 known limonoids, **4**–**17**. Compounds **1**–**3** were strong antifeedants against *Spodoptera* insects at 1 μg/cm² on leaf disks. The structures of compounds **1**–**3** were similar to those of the azadirachtins from *M. azadirachta indica*, particularly to 1-tigloyl-3-acetyl-11-methoxyazadirachtinin (**18**),[3a] except for the presence of an additional methyl group and some changes of substitution. This is the second isolation of the meliacarpinins.



R^3 : $X = COCH(CH_3)CH_2CH_3$
$Y = COCH(CH_3)_2$

5: $R^1 = OAc, R^2 = OAc, R^3 = X$
6: $R^1 = OAc, R^2 = OAc, R^3 = X, 1\alpha = OAc$
7: $R^1 = OAc, R^2 = OAc, R^3 = Y$
8: $R^1 = OAc, R^2 = OH, R^3 = X$
9: $R^1 = OAc, R^2 = H, R^3 = X$
10: $R^1 = OAc, R^2 = H, R^3 = Y$
11: $R^1 = OH, R^2 = OH, R^3 = X, 1\alpha = OAc$
12: $R^1 = H, R^2 = OH, R^3 = X$
13: $R^1 = H, R^2 = OAc, R^3 = X$
14: $R^1 = H, R^2 = OAc, R^3 = Y$
15: $R^1 = H, R^2 = H, R^3 = Y$

Meliacarpinin B (**1**), $C_{33}H_{44}O_{12}$, showed the presence of hydroxyl (3600-3300 cm^{-1}), ester (1740 cm^{-1}) and conjugated ester (1710 cm^{-1}) groups in the IR spectrum. The ^{13}C and ^{1}H NMR (at 27 and 45° C) data indicated that **1** contained 7 CH_3, 5 CH_2, 11 CH, 10 carbons (2 alkoxycarbonyl) not bonded to hydrogen and 2 protons due to OH groups. Furthermore, the NMR data suggested that the structure of **1** was almost superimposable on those of meliacarpinin A (**4**),[12] from *M. azedarach* collected at the Ryukyu Island. Especially, the chemical shifts for protons attached to carbons 7, 15 to 18 and 21 to 23 in **1** including 11-OMe, 11-CO_2Me and two OH groups were almost identical to the corresponding shifts in **4** except for the lack of one acetoxyl group and the presence of a tigloyl group instead of the cinnamoyl group in **4**. These data and the NOE enhancements (Figure 1) suggested the stereochemistry of the rings A-D in **1** also to be same as that of **4**.

The substitution pattern around the A-ring, namely, that **1** has a tigloyloxy group only at 3α, was deduced from the fact that a W-type long range coupling was observed between the 1α-H and 19-Ha signals at δ 1.38 and 4.06, and irradiation of the 5'-H_3 (2'-Me) signal of tigloyl at δ1.85 enhanced the signal due to 3β-H at δ5.00, which also showed a W-type long range coupling with 1β-H at δ1.59. The 4β-Me signal at δ 0.97 also showed a long range coupling with the 28α-H signal at δ3.56 and a NOE with the 19-Ha signal. From these results, compound **1** was elucidated as 1-deoxy-3-tigloyl-11-methoxymeliacarpinin.

The structure of meliacarpinin C (**2**), $C_{35}H_{46}O_{14}$, possessing an additional acetoxyl group at δ1.99 along with one tigloyl group, was readily suggested from the ^{1}H NMR spectrum, in which only one methine signal due to 1β-H showed a large down field shift to δ4.59 from δ1.38 in **1**. All the chemical shifts of another signals were superimposable on those of **1**, except that, by a conformation change of the 3α-tigloyl group or the ring A due to the introduction of 1α-acetoxyl group, down field shifts of the 5α- and 9α-H signals to δ 3.07 and 3.67 from δ2.73 and 3.16 in **1** and upfield shifts of the C-2, -5 and -9 signals to δ28.0, 35.1 and 47.9 from δ33.5, 39.7 and 54.8 in **1**, respectively, were observed in the ^{1}H and ^{13}C NMR spectra. Therefore, the structure **2** was secured for meliacarpinin C. A similar NOE observation in **2** to that in **1** also supported well the structure including the stereochemistry.

The third limonoid, meliacarpinin D (**3**), had the same molecular formura, $C_{35}H_{46}O_{14}$, as **2** and their NMR spectra were also superimposable on each other including acetyl and tigroyl signals except for some changes of the 1β-, 2α- and 9-H signals, which strongly suggested that they differed from one another only in the substituents at the ring A. That is to say, the fact that the compound **3** have 1α-tigloyloxy and 3α-acetoxyl groups was deduced from the presence of an additional NOE between 3'- and/or 2'-Me of tigloyl and 9-H. It is of interest that all the azadirachtin-type limonoids isolated from *M. azedarach* except for azadirachtin,[17] do not possess a 4β-CO_2Me but a 4β-Me group.

The antifeedant activities of the isolated limonoids, **1-17**, were tested by the conventional leaf disk method[16] against the larvae of *S. exigua* Hübner and *S. eridania* (Boisduval). The most potent was the meliacarpinins, **1-4**, which active at 50 ppm, corresponding to the concentration of 1 μg/cm^2 which may be less than those of the azadirachtins from the Indian neem tree *M. azadirachta indica* but belongs to the first class in the limonoids.[18] They were followed by the 12α-OH compounds of trichilins[19] and azedarachins with a 14,15-epoxide and a 19/29 acetal bridge, **8**, **11** and **12**: 200 ppm, and then the 12-deoxy type, **9** and **10**, and the 12-acetates, **5-7**, **13** and **14**: 400 ppm. Both nimbolinin B (**16**) and salannin (**17**) were poor antifeedants active at 1000 ppm.

Table 1. ^{13}C NMR data for the compounds **1-3**

C	**1**	**2**	**3**	C	**1**	**2**	**3**
1	24.9 t	71.1 d	71.0 d	19	71.0 t	70.7 t	70.9 t
2	33.5 t	28.0 t	28.6 t	20	86.2 s	86.2 s	86.6 s
3	70.7 d	70.3 d	70.5 d	21	109.4 d	109.3 d	109.5 d
4	42.7 s	42.7 s	42.7 s	22	108.0 d	107.9 d	108.3 d
5	39.7 d	35.1 d	35.5 d	23	146.0 d	145.8 d	146.1 d
6	71.0 d	71.1 d	71.5 d	28	76.8 t	76.5 t	76.9 t
7	84.3 d	83.8 d	83.6 d	29	18.4 q	18.0 q	18.7 q
8	51.0 s	51.2 s	51.7 s	30	17.2 q	17.8 q	18.0 q
9	54.8 d	47.9 d	48.2 d	CO_2Me	52.9 q	53.1 q	53.5 q
10	49.5 s	49.7 s	50.2 s		-	-	-
11	106.7 s	107.0 s	107.1 s	11-OMe	52.3 q	52.5 q	52.7 q
12	170.1 s	170.1 s	170.4 s	Ac	-	169.4 s	169.4 s
13	94.8 s	94.9 s	95.2 s		-	21.0 q	21.3 q
14	93.0 s	93.3 s	93.4 s	Tig: 1'	167.0 s	166.7 s	166.9 s
15	81.1 d	81.1 d	81.6 d	2'	128.5 s	128.4 s	128.8 s
16	29.8 t	29.7 t	29.9 t	3'	138.1 d	138.2 d	138.2 d
17	51.3 d	50.8 d	51.1 d	4'	14.5 q	14.4 q	14.5 q
18	26.3 q	25.9 q	26.9 q	5'	12.1 q	11.9 q	12.4 q

Table 2. ^{1}H NMR data for meliacarpinins A-D, (**4**) and (1-3)

H	**1**	**2**	**3**	**4**
1α	1.38 ddd(14.0, 14.0, 4.5)	-	-	-
1β	1.59 brd(14.0)	4.59 brd(2.9)	4.79 brt(2.7)	4.82 brt(2.6)
2α	2.18 m	2.34 dt(16.8, 2.5)	2.18 dt(16.8, 2.2)	2.36 dt(16.9, 2.5)
2β	1.87 m	2.06 dt(16.8, 3.2)	2.07 dt(16.8, 2.5)	2.08 dt(16.9, 2.5)
3β	5.00 brt(2.8)	4.97 brt(2.7)	4.92 brdd(3.5, 2.1)	4.94 brt(2.5)
5	2.73 d(12.8)	3.07 d(12.8)	3.01 d(13.2)	3.12 d(12.5)
6β	3.90 dd(12.9, 3.0)	3.96 dd(12.8, 2.6)	3.96 dd(12.8, 2.9)	3.99 dd(12.7, 2.7)
7β	4.52 d(2.9)	4.53 d(2.6)	4.51 d(2.9)	4.56 d(2.7)
9	3.16 s	3.67 s	3.55 brs	3.65 s
15	4.12 brs	4.14 br s	4.12 m	4.13 m
16a	2.20 m	2.22 ddd(13.2, 6.3, 2.4)	2.17 m	2.17 m
16b	1.88 m	1.87 dd(13.4, 1.0)	1.86 dd(11.7, 1.5)	1.87 m
17	2.16 m	2.17 brdd(6.1, 1.09)	2.14 m	2.17 m
18(Me)	1.50 s	1.61 s	1.50 s	1.57 s
19a	4.06 brd(8.6)	4.16 brd(9.2)	4.16 d(9.2)	4.18 d(9.5)
19b	4.96 d(8.8)	3.86 d(9.2)	3.89 d(9.2)	3.91 d(9.5)
21	5.63 s	5.64 s	5.64 s	5.64 s
22	4.88 d(2.9)	4.89 d(3.0)	4.87 d(2.9)	4.89 d(2.9)
23	6.38 d(2.9)	6.39 d(3.0)	6.38 d(2.9)	6.39 d(2.9)
28α	3.56 brd(7.7)	3.58 d(8.1)	3.59 d(7.8)	3.64 d(7.3)
28β	3.52 d(7.7)	3.55 d(8.1)	3.57 d(7.7)	3.59 d(7.3)
29(Me)	0.97 s	0.99 s	1.00 s	1.01 s
30(Me)	1.53 s	1.58 s	1.55 s	1.63 s
14-OH	4.20 s	4.14 s	4.28 s	4.24 s
20-OH	6.15 s	6.16 s	6.11 s	6.15 s
11-OMe	3.42 s	3.38 s	3.37 s	3.38 s
12-OMe	3.81 s	3.74 s	3.72 s	3.73 s
Ac	-	1.99 s	1.96 s	1.91 s
Tig: 3'	6.88 qq(7.0, 1.5)	6.91 qq(8.3, 1.5)	6.92 qq(7.0, 1.5)	
4' (Me)	1.83 dq(7.3, 1.1)	1.82 dq(8.3, 1.3)	1.80 dq(7.3, 1.1)	
5' (Me)	1.85 dq(1.5, 1.1)	1.83 dq(1.5, 1.3)	1.85 dq(1.5, 1.1)	
.Cin: 2'				6.43 d(16.0)
3'				7.73 d(16.0)
ph-H				7.41-7.52

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were measured at 400 and 100 MHz in $CDCl_3$ on a JEOL FX-400 spectrometer. IR (KBr) and UV (in MeOH) spectra were recorded on JASCO FT/IR 5300 and Shimazu UV-21-A spectrophotometers. Optical and CD spectra were measured in MeOH using a JASCO J-20A spectropolarimeter. HPLC was performed on Waters μPorasil and μBondasphere columns by using 0.5-2.0% MeOH-CH_2Cl_2 and 20-50% H_2O-MeOH as solvents, respectively.

Plant material. The root bark was collected in October 1992 at Guangzhou in China.

Extraction and isolation. The dried root bark (375 g) was extracted with Et_2O to yield 3.1 g of material which was dissolved in 13 ml Et_2O and then added to the same vol. of hexane to give 975 mg of a ppt. It was flash chromatographed on silica gel with 0.5-10% MeOH-CH_2Cl_2, and the limonoid frs eluted with 1-2% MeOH-CH_2Cl_2 were rechromatographed on a flash column with 20-0% hexane-Et_2O. Each limonoid fr. was separated through HPLC using normal, and reversed columns, to give **1** (0.8 mg), **2** (2.2 mg) and **3** (6.8 mg), and 14 known limonoids, **4** (0.4 mg), **5** (5.8 mg), **6** (1 mg), **7** (2.6 mg), **8** (1 mg), **9** (7.5 mg), **10** (3.3 mg), **11** (0.5 mg), **12** (1 mg), **13** (1 mg), **14** (2.5 mg), **15** (0.5 mg), **16** (1 mg) and **17** (3.5 mg).

Meliacarpinin B (1). An amorphous powder, $C_{33}H_{44}O_{12}$; HRFABMS m/z: 655.2744 $(M+Na)^+$, + 1.3 mmu; $[\alpha]_D^{22}$ -6.7° (c 0.06); IR ν_{max} cm^{-1}: 3600-3300, 1740, 1710, 1655 and 1625; UV λmax nm (ϵ) : 270 (1500) and 213 (4500); CD: $\Delta\epsilon_{233}$ +13 and $\Delta\epsilon_{209}$ -45.

meliacarpinin C (2). An amorphous powder, $C_{35}H_{46}O_{14}$; HRFABMS m/z: 713.2820 $(M+Na)^+$, +3.5 mmu; $[\alpha]_D^{22}$ +9.1° (c 0.22); IR νmax cm^{-1}: 3600-3300, 1740, 1710, 1655 and 1625; UV λmax nm (ϵ): 283 (400) and 217 (10500); CD: $\Delta\epsilon_{227}$ +2.51 and $\Delta\epsilon_{211.5}$ -7.54.

meliacarpinin D (3). An amorphous powder, $C_{35}H_{46}O_{14}$; HRFABMS m/z: 713.2831 $(M+Na)^+$, +4.6 mmu; $[\alpha]_D^{22}$ -8.3° (c 0.52) ; IR ν_{max} cm^{-1}: 3600-3300, 1740, 1710, 1655 and 1625; UV λmax nm (ϵ): 215 (9600); CD: $\Delta\epsilon_{240}$ +6.1 and $\Delta\epsilon_{215}$ -25.6.

meliacarpinin A (4). An amorphous powder, $C_{39}H_{46}O_{14}$; CIMS m/z: 739 $(M+1)^+$; UV λmax nm (ϵ) : 277 (1500) and 215 (6400).

Bioassay of the antifeedants. The antifeedant potential of the isolated compounds was assessed by presenting them on leaf disks of a Chinese cabbege to the third instar larvae of *spodoptera eridania* and *S. exigua* Hübner (Boisduval), and visually comparing the treated and untreated leaves eaten by the larvae. The larvae were placed in a Petri dish with the five treated leaf disks with sample and the five untreated disks as controls. The feeding bioassays terminated after the larvae had eaten approximately 50% of one of the disks, which took 6-24 hr. This choice test was done 50, 100, 200, 300, 400, 500 and 1000 ppm concentrations to determine minimum inhibitory concentration for each of the compounds.

Antifeedant activities. Effective conc.; meliacarpinins B (**1**), C (**2**), D (**3**) and A (**4**): 50 ppm. Trichilin B (**8**), aphanastatin (**11**) and azedarachin A (**12**): 200 ppm. 12-*O*-acetyl (**5**) and 1,12-di-*O*-acetyltrichilin B (**6**), trichilins H (**7**) and D (**9**), meliatoxin A_2 (**10**) and 12-*O*-acetylazedarachins A (**13**), B(**14**) and azedarachin C (**15**): 400ppm. Nimbolinin B (**16**) and salannin (**17**): 1000 ppm.

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