

**MELIANOLIDE, A NEW LIMONOID OF BIOGENETIC INTEREST,
FROM CHINESE *MELIA AZEDARACH* L.**

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Abstract — Melianolide, a new limonoid of biogenetic interest, was isolated along with salannal and four known ring C-seco limonoids, nimbolinin B, salannin, deacetylsalannin and nimbolidin B, from the root bark of Chinese *Melia azedarach* L. (Meliaceae). The structure is proposed on the basis of spectral data.

Limonoids from *Melia* species are attracting considerable interest, because of a variety of structures and insect feeding inhibitory properties.¹ In a series of experiments, we have isolated four azadirachtin-type meliacarpinins,² and eleven intact apo-euphol limonoids with C-19/C-29 bridged acyl acetals, trichilins³ and azedarachins,⁴ as insect "antifeedant" from chinese *Melia azedarach* L. In the continuous study of the plant, we isolated a new biogenetically interesting limonoid, named melianolide (**1**), along with salannal (**2**)⁵ and four known ring C-cleaved limonoids, nimbolinin B (**3**),⁶ salannin (**4**),⁷ deacetylsalannin (**5**)⁷ and nimbolidin B (**6**).⁶ Melianolide (**1**) is a new type limonoid having an acetal ring system. In this paper we intend to report the structure elucidation of **1** by spectroscopic means, and a biogenesis and antifeedant activities of the isolated limonoids by a conventional leaf disk method⁸ against a Japanese

pest insect *Spodoptera eridania* (Boisduval).

From the ether extract of the air-dried root bark (1.5 kg) of *M. azedarach*, compounds **1** (1.2 mg), **2** (0.7 mg), **3** (1.2 mg), **4** (3.5 mg), **5** (0.8 mg) and **6** (0.8 mg) were isolated by flash chromatography on silica gel with 1.0% MeOH-CH₂Cl₂ and 20% hexane-Et₂O solvent systems followed by hplc using normal and reverse phase columns with 0.5-2% MeOH-CH₂Cl₂ and 25-40% H₂O-MeOH as the solvents. Four known compounds (**3-6**) were identified as nimbolinin B, salannin, deacetylsalannin and nimbolidin B, respectively, by comparison of their nmr spectra with the published data. Compounds (**4**) and (**5**) are popular C-seco limonoids from Meliaceae plants, and **3** and **6** have been found in *M. azedarach* from the former Yugoslavia.⁶

Melianolide (**1**) exhibited the following spectral data: $[\alpha]_D^{26} -2^\circ$ (c 0.06, MeOH); ir: 3550-3250, 1735, 1710, 1640 and 1616 cm⁻¹; uv: 219 nm(ϵ 9000); and HRFABms: m/z 643.3129 [M+H]⁺ (Δ +1.1 mmu), corresponding to formula C₃₅H₄₆O₁₁ (13 unsaturations). The ¹³C and ¹H nmr (Table 1) indicated that **1** contained 8 CH₃, 4 CH₂, 13 CH, 10 carbons not bonded hydrogen, including three C=C double bond, and one proton due to OH group. Furthermore, the nmr spectra showed the presence of a β -furyl moiety and two acetyl and one tigloyl groups. The structure of **1** including its stereochemistry was elucidated based on the nmr data and decoupling and nOe experiments. The presence of acetal and alkoxyethylene carbons at δ 96.4d (C-12) and 78.8t (C-28), respectively, suggested that **1** was a ring C-seco limonoid similar to nimbolinins except for the lack of 13,14-double bond. The 7 β -H signal at δ 4.48 (d, J=3.9 Hz), coupling with the 6-H signal at δ 3.93 (dd, J=12.3 and 3.9 Hz), was changed to a more sharp doublet by the addition of D₂O, which showed that C-7 was not formed an ether linkage with C-15 different from that in **2** and **4**, but had an OH group. The presence of a proton triplet at δ 5.43 assigned to H-12, strongly

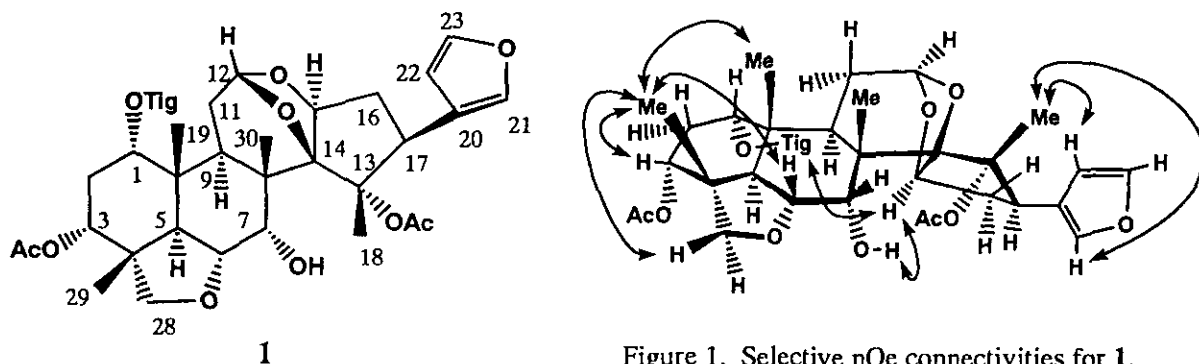


Figure 1. Selective nOe connectivities for **1**.

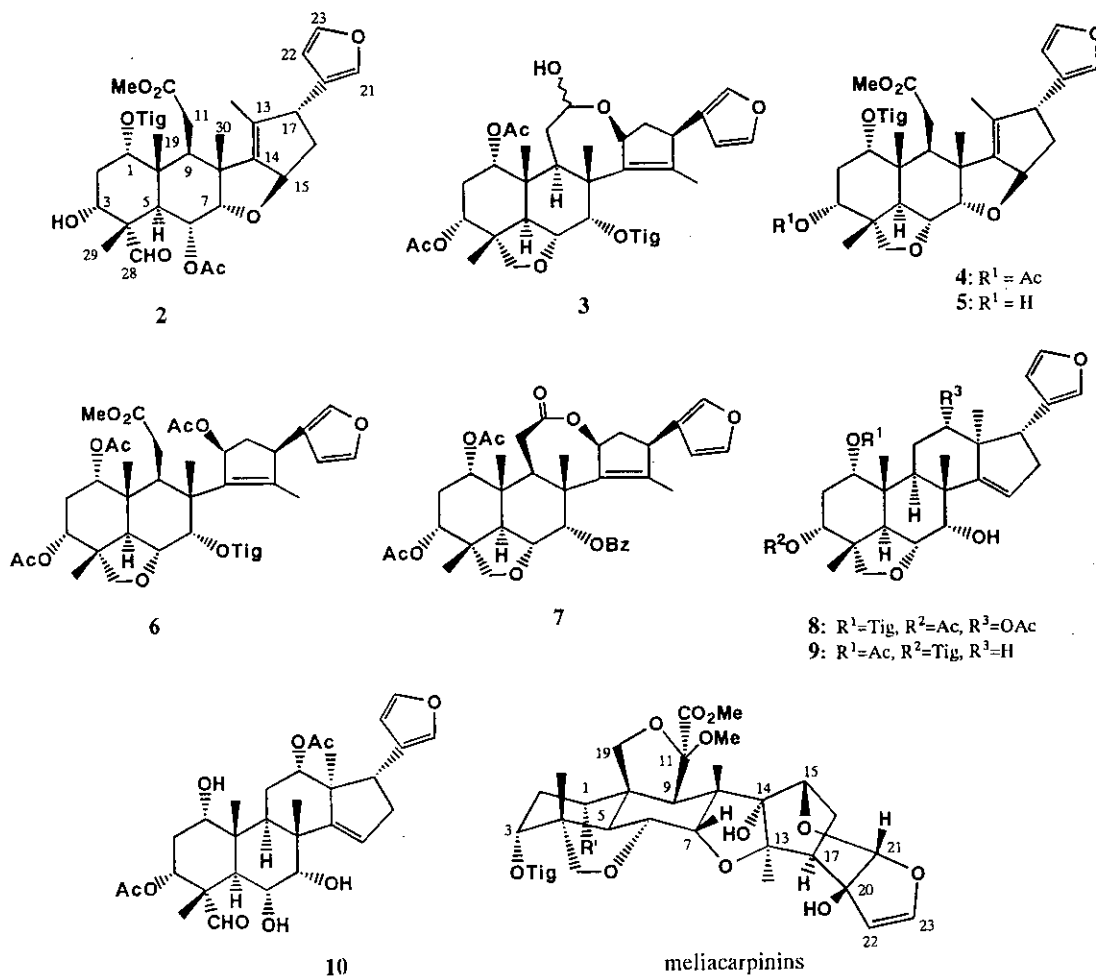
Table. 1 ^1H and ^{13}C Nmr Data for Melianolide (1) in CDCl_3

position	^1H	^{13}C	position	^1H	^{13}C
1	4.70 t(2.9)	71.5 d	17	3.08 ddd(9.7,5.2,1.3)	44.9 d
2	2.11 br dt(4.0, 2.9)	28.1 t	18	1.35 br s	16.6 q
	2.12 br dt(4.0, 2.9)		19	0.97 s	16.6 q
3	4.90 t(2.8)	67.0 d	20		123.6 s
4		42.7 s	21	7.20 m	142.4 s
5	2.67 d(12.5)	39.0 d	22	6.42 d(1.3)	111.1 d
6	3.93 dd(12.5,3.9)	71.9 d	23	7.36 dd(1.7,1.3)	139.6 d
7	4.48 d(3.9)	74.6 d	28 α	3.65 br d(7.5)	78.8 t
8		44.9 s	β	3.62 d(7.5)	
9	2.90 dd(12.8,7.0)	35.6 d	29	1.25 br s	20.4 q
10		39.1 s	30	1.45 s	20.5 q
11 α	1.61 m	32.3 t	OH	2.50 s	
β	2.36 ddd(12.8,12.0,7.7)		OAC	2.02 s, 2.09 s	21.0 q, 21.6 q
12	5.43 t(7.7)	96.4 d			168.8 s, 170.2 s
13		81.3 s	OTig: 1'		167.3 s
14		77.7 s	2'		129.0 s
15	4.41 m	80.3 d	2'-Me	1.88 dq(1.4,1.1)	12.2 q
16 α	1.54 m	33.7 t	3'	6.96 qq(7.2,1.4)	138.2 d
β	1.71 br dd(13.5,5.2)		3'-Me	1.83 dq(7.2,1.1)	14.7 q

Measured at 400 and 100 MHz. Coupling constant (Hz) in parenthesis.

suggested that C-12 should be constructed an acetal ring with C-14 (δ 77.7 s) and 15 (δ 80.3 d). The presence of α -tigloyloxy group was deduced from the high field shifts of the 9- and 11 α -H signals to δ 2.90 and 1.61 from δ 3.40 and 2.66 in ohchinolide A (7)⁶ like in trichilinins, i.e. δ 2.80 and 1.08 in 8 and δ 3.18 and 2.22 in 9,⁹ attributable to a shielding effect of the α -tigloyl group.^{6a} NOe observation between the 3'-Me and 15-H signals, furthermore, supported the α -tigloyloxy group. The α configuration of 15-H was elucidated from the small coupling constant with 16-H₂ similar to that in meliacarpinins² and NOe correlation of the 15-H with the 7 α -OH signal. On the other hand, the β orientation of 13-Me was confirmed from a weak coupling with the 17-H signal and NOe observation between the 13-Me group and the 21- and 22-furan protons. A similar W-type long range coupling of methine or one of methylene protons with methyl group was also observed between H-9 and 10-Me and H-28 α and 4 β -Me.^{1b,9} Remaining one acetoxyl group was, consequently, located at C-13 α .

It seems that melianolide (1) is situated in a position to link ring C-seco limonoids such as nimbolin B (3) to meliacarpin class. On the other hand, salannal (2) should have been



produced from an intact apo-euphol limonoid such as sendanal (7)¹⁰ by a Grob type olefin-forming fragmentation¹¹ via a ring C cleaved 12-aldehyde with 13,14-double bond and 15-hydroxyl group¹¹ and by subsequent ether ring formation between C-7 and 15 hydroxyl groups. Further ether formation between C-28 and C-6 would yield salannin class C-seco limonoids. On the other hand, a C-28/C-6 ether ring formation in a hydroxy-aldehyde Grob fragment would give nimbolinin or nimbolidin type compounds.

The antifeedant activity of the isolated limonoids (3-6) against the third-instar larvae of a Japanese pest insect *Spodoptera eridania* (Boisduval) was tested by a conventional leaf disk method.⁸ These compounds showed only weak activities, 3-5: 1000 ppm and 6: 500 ppm, compared to that of meliacarpinins: 50 ppm, corresponding to the concentration of 1 $\mu\text{g}/\text{cm}^2$. Compounds (1) and (2) were not tested because they had decomposed during spectral studies before the test.

EXPERIMENTAL

^1H and ^{13}C nmr were measured in CDCl_3 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 and $[\text{C}_\text{D}]$ were measured in MeOH using a JASCO J-20 A spectrometer.

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

Extraction and isolation. The dried bark (1.5 kg) was extracted with ether (20 l) at 15°C for 2 weeks, to yield 12.8 g of an extract, which was dissolved in 50 ml of ether and then added to the same volume of hexane to give 3.9 g of a precipitate. It was flash chromatographed on silica gel with 0.5-10% MeOH- CH_2Cl_2 , and the limonoid fractions eluted with 1.0% MeOH- CH_2Cl_2 were rechromatographed on a flash column with 50-0% hexane-ether. Each limonoid fraction eluted with 20% hexane-ether was separated through hplc, $\mu\text{Porasil}$ and $\mu\text{Bondasphere}$ semiprep columns, with 0.7-2.0% MeOH- CH_2Cl_2 and 20-40% H_2O -MeOH as the solvents, respectively, to give the following limonoids as well as six trichilins, three azedarachins and four meliacarpinins reported previously: **1** (1.2 mg), **2** (0.7 mg), **3** (1.2 mg), **4** (3.5 mg), **5** (0.8 mg) and **6** (0.8 mg).

Melianolide (1). An amorphous powder, $\text{C}_{35}\text{H}_{46}\text{O}_{11}$; $[\alpha]_\text{D}^{22} -2^\circ$ (c 0.06); uv 219 nm(ϵ 9000); ir 3550-3250, 1735, 1710, 1640, and 1616 cm^{-1} ; negative FABms m/z 641 $[\text{M}-\text{H}]^-$ and HRFABms m/z 643.3129 $[\text{M}+\text{H}]^+$ (Δ +1.1 mmu); $[\text{C}_\text{D}]_{215} -5.7$.

Known limonoids. Salannal (**2**), $\text{C}_{34}\text{H}_{44}\text{O}_{13}$; $[\alpha]_\text{D}^{20} +67^\circ$ (c 0.05); FABms m/z 613 $[\text{M}+\text{H}]^+$.

Nombolinin B (**3**), $\text{C}_{35}\text{H}_{46}\text{O}_{10}$; $[\alpha]_\text{D}^{22} -10^\circ$ (c 0.05); $[\text{C}_\text{D}]_{223} -7.2$. Salannin (**4**), $\text{C}_{34}\text{H}_{44}\text{O}_9$; $[\alpha]_\text{D}^{22} +134^\circ$ (c 0.05); $[\text{C}_\text{D}]_{216} +17$. Deacetylsalannin (**5**), $\text{C}_{32}\text{H}_{42}\text{O}_8$; $[\alpha]_\text{D}^{24} +54^\circ$ (c 0.1). Nimbolidin B (**6**) $\text{C}_{38}\text{H}_{50}\text{O}_{12}$; $[\alpha]_\text{D}^{26} -7^\circ$ (c 0.1).

Bioassay of the antifeedants. The antifeedant activity of the isolated compounds was tested by a conventional leaf disk method⁷ against the third-instar larvae of *S. eridania* (Boisduval). Five disks of Chinese cabbage treated with the sample were arranged with another five control disks immersed in acetone alone in a Petri dish, ten larvae were placed in the center, and the score for the treated and untreated leaves eaten by the larvae in 10-24 h was evaluated. From these choice tests at 300, 400, 500 and 1000 ppm concentrations, the minimum inhibitory concentrations were determined.

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