ISOLATION OF A NEW INSECT ANTIFEEDING AZADIRACHTIN DERIVATIVE FROM OKINAWAN *MELIA AZEDARACH* LINN

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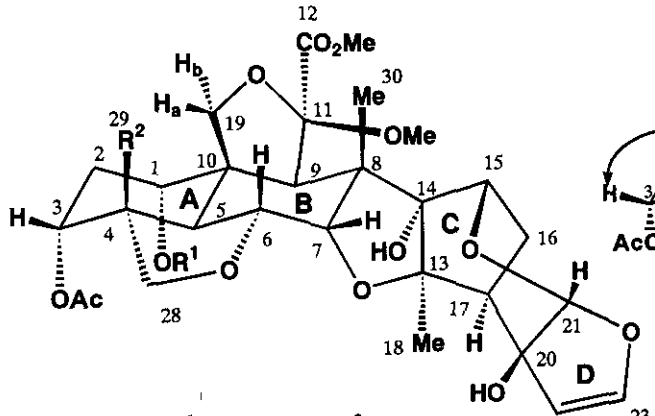
Abstract - A new azadirachtin-type insect antifeedant, 1-cinnamoyl-3-acetyl-11-methoxymeliacarpinin, was isolated from the stem bark of Okinawan *Melia azedarach* Linn.

The neem tree *Melia azadirachta indica* Juss (Meliaceae) and the related tree *Melia azedarach* L. are attracting considerable interest, particularly because of their insect antifeedant properties. The most potent constituents are azadirachtin[1] and related compounds.[2,3] Herein we report the isolation of a new limonoid antifeedant, 1-cinnamoyl-3-acetyl-11-methoxymeliacarpinin (1), structurally related to azadirachtin. This is the second isolation of azadirachtin derivative from *M. azedarach*.[2b] During the investigation of limonoid antifeedants from Meliaceae plants,[4] we got a new azadirachtin derivative (1) along with toosendanin[5] (chuanliansu[6]) from the stem bark of Okinawan *M. azedarach* L. They are antifeedants active against the voracious Japanese pest insect *Spodoptera exigua* Hübner (Boisduval).

Compound (1), 0.6 mg, was isolated from the ether extract of the stem bark (1.2 kg, yield 5×10^{-5} %) by very carefully combined use of normal- and re-



1: R^1 = cinnamoyl, R^2 = CH_3
2: R^1 = tigloyl, R^2 = CO_2CH_3

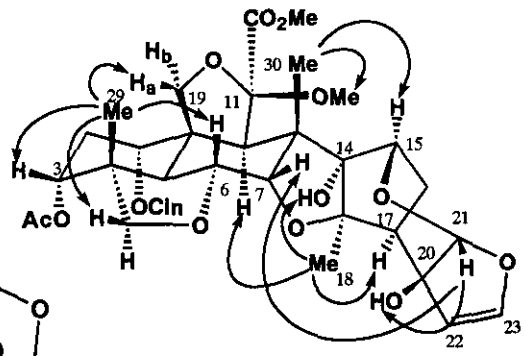


Figure 1. Selected NOE connectivities for **1**

versed-phase hplc. The molecular formula $C_{39}H_{46}O_{14}$ of **1** was derived from the SI-ms (m/z 739 $[M+1]^+$). The ^1H nmr data for **1** revealed a strong resemblance to 1-tigloyl-3-acetyl-11-methoxyazadirachtinin (**2**)[3a] except for the presence of an additional methyl and a cinnamoyl groups instead of the lack of one methoxycarbonyl and the tigloyl groups in **2**. Especially, the chemical shifts for protons attached to carbons 7 to 18 and 21 to 23 in **1** including 11-OMe, 12-CO_2Me and two OH groups were almost identical to the corresponding shifts in **2**. These data and the NOE enhancements (Figure 1) suggested the stereochemistry of the B, C and D rings in **1** to be same with that of **2**. The fact that the 4β-methoxycarbonyl group in **2** changed to methyl group in **1** was deduced from the chemical shifts of 3β-, 6β- and 28β-H. The high shifts of δ 4.94, 3.99 and 3.59 compared to those (δ 5.48, 4.39 and 4.03) of **2**, respectively, could be attributed to the removal of the anisotropic effect of the 29-carbonyl group. The presence of a 4β-Me group was also supported from a long range coupling with 28α-H at δ 3.64 and NOEs between 29-H and 3β-, 6β-H and 19-Ha.

On the other hand, the substitution pattern around the A-ring, namely, that **1** has 1-cinnamoyloxy and 3-acetoxy groups, was deduced from the chemical shifts of 1β- and 28α-H at δ 4.82 and 3.64 very similar to those (δ 4.81 and

3.66) in **2**. In an azadirachtin derivative, it has been observed that the 28α-H signal is shifted down-field to ca. 0.1 ppm lower by the introduction of cinnamoyloxy group instead of the 3α-acetoxy group.[3a]

The compound (**1**) showed an antifeedant activity at 50 ppm, corresponding to the concentration of 1μg/cm^2, by the conventional leaf disk method[7] against the larvae of *Spodoptera exigua* Hübner (Boisduval). The activity is the most potent in the limonoids isolated by us.[5] Although it may be weaker than those of azadirachtin or 1-tigloyl-3-acetyl-11-methoxyazadi-

Table 1. ^{1}H Nmr Data for **1** and **2**[3a] (in $CDCl_3$)

H	**1** δ Mult (J/Hz)	**2** δ Mult (J/Hz)	H	**1** δ Mult (J/Hz)	**2** δ Mult (J/Hz)
1	4.82 br t (2.6)	4.81 dd (3.1, 2.9)	21	5.64 s	5.64 s
2α	2.36 dt (16.9, 2.4)	2.28 ddd (16.7, 2.9, 2.3)	22	4.89 d (2.9)	4.88 d (2.9)
2β	2.08 dt (16.9, 2.5)	2.13 ddd (16.7, 3.3, 3.1)	23	6.39 d (2.9)	6.39 d (2.9)
3	4.94 br t (2.5)	5.48 dd (3.3, 2.4)	28α	3.64 d (7.3)	3.66 d (8.8)
5	3.12 d (12.5)	3.16 d (12.7)	28β	3.59 d (7.3)	4.03 d (8.8)
6	3.99 dd (12.7, 2.7)	4.39 dd (12.7, 3.2)	29	1.01 s	
7	4.56 d (2.7)	4.53 d (3.2)	30	1.63 s	1.57 s
9	3.65 s	3.56 s	14-OH	4.24 s	4.33 s
15	4.13 m	4.13 m	20-OH	6.15 s	6.07 s
16a	2.17 m	2.15 m	11-OMe	3.38 s	3.37 s
16b	1.87 m	1.85 m	12-OMe	3.73 s	3.72 s
17	2.17 m	2.12 m	29-OMe		3.77 s
18	1.57 s	1.50 s	Ac	1.91 s	1.99 s
19a	4.18 d (9.5)	4.21 d (9.7)	cinnamoyl		tigloyl
19b	3.91 d (9.5)	3.73 d (9.7)	2'	6.43 d (16.0)	3' 6.93 qq (7.0, 1.5)
			3'	7.73 d (16.0)	4' 1.81 dq (7.0, 1.1)
			ph-H	7.52 - 7.41	5' 1.85 dq (1.1, 1.5)

rachtinin (2),[4b] it is apparently stronger than those (200 - 400 ppm) of the second class limonoids[4a] such as trichilins[8] from African *Trichilia roka* and sendanin[9,10] from Japanese *Melia azedarach* L. with a 14,15-epoxide and a 19/29 lactol bridge.

EXPERIMENTAL

^{1}H Nmr (400 MHz) spectra were measured in $CDCl_3$ on a JEOL FX-400 spectrometer. Uv spectrum was recorded in MeOH on a Shimazu UV-210A spectrophotometer. Hplc was performed on WATERS μ Porasil and μ Bondasphere columns by using 0.7-1.5% MeOH-$CHCl_3$ and 30-40% H_2O-MeOH as solvent, respectively.

Plant material. Stem bark of the plant was collected in October 1991 at Haibaru near Naha, Okinawa.

Extraction and isolation of 1. The stem bark (1.2 kg) of *M. azedarach* L. was extracted with MeOH (8 l) for five weeks at room temperature. After concn to 200 ml, water (300 ml) was added and the solution was extracted with hexane, ether, ethyl acetate and *n*-BuOH, successively. The ether extract (4.5 g) was chromatographed on SiO_2 with 2% MeOH-$CHCl_3$, and an active fraction (298 mg) was rechromatographed with ether to give 65 mg of an active fraction. Final purification was done by combined use of normal- and reversed-phase hplc to give 0.6 mg of **1**.

1: powder; SI-ms m/z 739 $(M+1)^+$, 721 $(M+1-H_2O)^+$ and 131 $(C_9H_7O)^+$; uv 277 and 215 nm (ε 15000 and 6400); ^{1}H nmr: Table 1.

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