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

Ruo Chun Huang, Yuji Minami, Fumio Yagi, Yumiko Nakamura,[b] Noboru Nakayama,[b] Kenjiro Tadera, and Munehiro Nakatani*[a]

Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University, Korimoto, Kagoshima 890, Japan

[a] Department of Chemistry, Faculty of Science, Kagoshima University, Korimoto, Kagoshima 890, Japan

[b] Nippon Roche Research Center, Kajiwara, Kamakura 247, Japan

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, trichilins[3] 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]

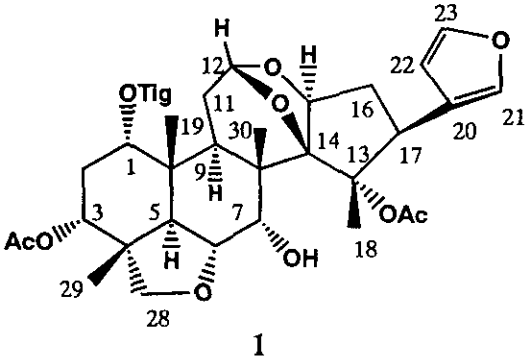
Melianolide (**1**) is a new type limonoid having an acetal ring system. In this paper we intend to report the structure elucidation of **1** by spectroscopic means, and a biogenesis and antifeedant activities of the isolated limonoids by a conventional leaf disk method[8] against a Japanese

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-CH_2Cl_2 and 20% hexane-Et_2O solvent systems followed by hplc using normal and reverse phase columns with 0.5-2% MeOH-CH_2Cl_2 and 25-40% H_2O-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° (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 $C_{35}H_{46}O_{11}$ (13 unsaturations). The ^{13}C and ^1H nmr (Table 1) indicated that **1** contained 8 CH_3, 4 CH_2, 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 alkoxymethylene 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 7β-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 D_2O, 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



1

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

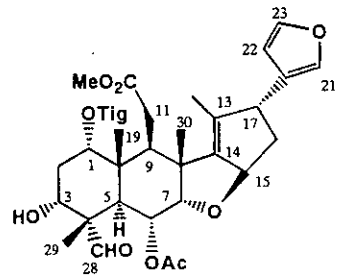
Table. 1 ^{1}H and ^{13}C Nmr Data for Melianolide (1) in $CDCl_3$

position	^{1}H	^{13}C	position	^{1}H	^{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

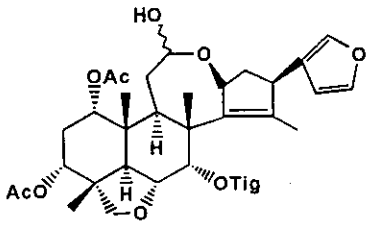
Measured at 400 and 100 MHz. Coupling constant (Hz) in parenthesis.

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 lα-tigloyloxy group was deduced from the high field shifts of the 9- and 11α-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 lα-tigloyl group.[6a] NOe observation between the 3'-Me and 15-H signals, furthermore, supported the lα-tigloyloxy group. The α configuration of 15-H was elucidated from the small coupling constant with 16-H_2 similar to that in meliacarpinins[2] and nOe correlation of the 15-H with the 7α-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-28α and 4β-Me.[1b,9] Remaining one acetoxyl group was, consequently, located at C-13α.

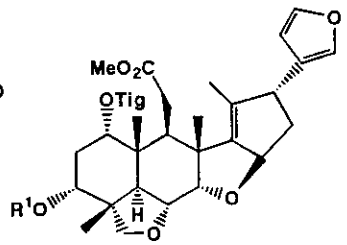
It seems that melianolide (**1**) is situated in a position to link ring C-seco limonoids such as nimbolinin B (**3**) to meliacarpinin class. On the other hand, salannal (**2**) should have been



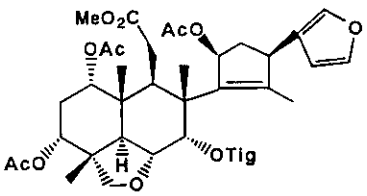
2



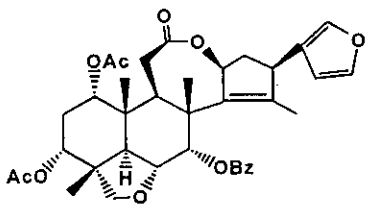
3



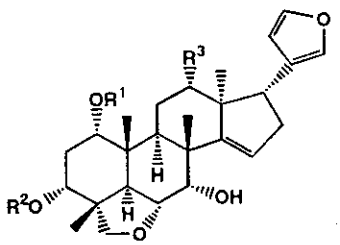
4: R^1 = Ac
5: R^1 = H



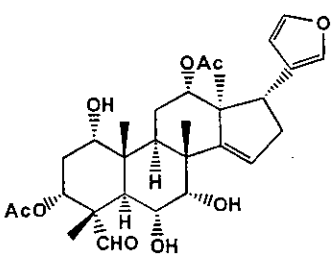
6



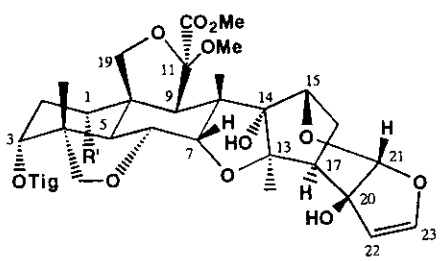
7



8: R^1=Tig, R^2=Ac, R^3=OAc
9: R^1=Ac, R^2=Tig, R^3=H



10



meliacarpinins

produced from an intact apo-euphol limonoid such as sendanal (7)[10] by a Grob type olefin-forming fragmentation[11] *via* a ring C cleaved 12-aldehyde with 13,14-double bond and 15-hydroxyl group[11] 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 μg/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 Cd were measured in MeOH using a JASCO J-20 A spectrometer.

Plant material. The root bark was collected in October 1992 at Guangzou, 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, μPorasil and μBondasphere 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, $C_{35}H_{46}O_{11}$; $[\alpha]_D^{22}$ -2° (c 0.06); uv 219 nm(ε 9000); ir 3550-3250, 1735, 1710, 1640, and 1616 cm^{-1}; negative FABms m/z 641$[M-H]^-$ and HRFABms m/z 643.3129$[M+H]^+$ (Δ +1.1 mmu); cd $\Delta\varepsilon_{215}$ -5.7.

Known limonoids. Salannal (**2**), $C_{34}H_{44}O_{13}$; $[\alpha]_D^{20}$ +67° (c 0.05); FABms m/z 613$[M+H]^+$. Nombolinin B (**3**), $C_{35}H_{46}O_{10}$; $[\alpha]_D^{22}$ -10° (c 0.05); cd $\Delta\varepsilon_{223}$ -7.2. Salannin (**4**), $C_{34}H_{44}O_9$; $[\alpha]_D^{22}$ +134° (c 0.05); cd $\Delta\varepsilon_{216}$ +17. Deacetylsalannin (**5**), $C_{32}H_{42}O_8$; $[\alpha]_D^{24}$ +54° (c 0.1). Nimbolidin B (**6**) $C_{38}H_{50}O_{12}$; $[\alpha]_D^{26}$ -7° (c 0.1).

Bioassay of the antifeedants. The antifeedant activity of the isolated compounds was tested by a conventional leaf disk method[7] 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.

ACKNOWLEDGEMENT

We wish to thank Mr. K. Takesaki, Kagoshima Prefectural Agricultural Experiment Station, for the supply of the insects.

REFERENCES

1. D.E. Champagne, O. Koul, M.B Isman, G.G.E. Scudder, and G.H.N. Towers, *Phytochemistry*, 1992, **31**, 377.
2. a) M. Nakatani, S. Arikawa, H. Okamura, and T. Iwagawa, *Heterocycles*, 1994, **35**, 327;
   b) M. Nakatani, R.C. Huang, H. Okamura, T. Iwagawa, K. Tadera, and H. Naoki, *Teterahedron*, 1995, **51**, 11731.
3. M. Nakatani, R.C. Huang, H. Okamura, H. Naoki, and T. Iwagawa, *Phytochemistry*, 1994, **36**, 39.
4. a) R.C. Huang, H. Okamura, T. Iwagawa, and M. Nakatani, *Bull, Chem. Soc. Jpn.*, 1994, **67**, 2468; b) R.C. Huang, H. Okamura, T. Iwagawa, K. Tadera, and M. Nakatani, *Phytochemistry*, 1995, **38**, 593.
5. M. Nakatani, R.C. Huang, H. Okamura, T. Iwagawa, and K. Tadera, *Chem. Lett.*, **1995**, 995.
6. W. Kraus and M. Bokel, *Chem. Ber.*, 1981, **114**, 267.
7. a) R. Henderson, R. McCrindle, A. Melera, and K.H. Overton , *Tetrahedron*, 1968, **24**, 1525;
   b) I. Kubo, A. Matsumoto, and T. Matsumoto, *Tetrahedron*, 1986, **42**, 489.
8. K. Wada and K. Munakata, *J. Agr. Food Chem.*, 1968, **17**, 471.
9. J-Bo Zhou, H. Okamura, T. Iwagawa, Y. Nakamura, N. Nakayama, K. Tadera, and M. Nakatani, *Heterocycles*, 1995, **41**, 2795.
10. M. Ochi, H. Kotsuki, and T. Tokoroyama, *Chem. Lett.*, **1978**, 621.
11. C.A. Grob and P.W. Schiess, *Angew. Chem., Int. Ed. Engl.*, 1967, **6**, 1.
12. D.E.U. Ekong, C.O. Fakunle, A.K. Fakunle, A.K. Fasina, and J.I. Okogun, *J. Chem. Soc., Chem. Comm.*, **1969**, 1166.

Received, 21st March, 1996