Pergamon

PII: S0031-9422(96)00353-6

Phytochemistry, Vol. 43, No. 3, pp. 581–583, 1996

0031-9422/96 $15.00 + 0.00

LIMONOIDS FROM *MELIA AZEDARACH*

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(*Received in revised form* 7 *May* 1996)

Key Word Index—*Melia azedarach*; Meliaceae; ring-C seco limonoids; insect antifeedants; salannal; meliacarpinin E; salannin; deacetylsalannin; nimbolinin B; nimbolidin B.

Abstract—A biogenetically interesting ring-C seco limonoid, salannal, and a potent insect antifeedant, meliacarpinin E, were isolated from the root bark of Chinese *Melia azedarach*, along with four known seco limonoids, salannin, deacetylsalannin, nimbolinin B and nimbolidin B. Their structures were elucidated by spectroscopic studies and their antifeedant properties were examined with the larvae of *Spodoptera eridania*. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Limonoid constituents of *Melia azedarach* L., as well as the neem tree *M. azadirachta* indica, are attracting considerable interest because of their variety of structures and biological activities. *M. azedarach* is a large tree, native of Persia, India and China, that is now naturalized in a number of continents including Africa, Australia and the Americas, and there are many reports to data on the limonoid constituents. We have isolated 12 new limonoids, along with seven known compounds, from Okinawan and Chinese plants, in which azadirachtin related meliacarpinins [1] and C-19/C-29 bridged acyl acetals [2], azedarachins and trichilins, are particularly interesting because of their potent insect antifeedant activities [3].

In our continuing study of the minor limonoid constituents of the Chinese plant collected at Guangzhou, we have isolated two new ring-C seco limonoids, salannal and meliacarpinin E, along with four known seco limonoids, salannin, deacetylsalannin [4, 5], nimbolinin B and nimbolidin B [6]. Salannal is the first ring C-seco limonoid having a 4α-formyl group and it could be a precursor of the salannin class of compounds. On the other hand, meliacarpinin E shows a potent insect antifeedant property, as found with other meliacarpinins. 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 disk method [7] against the larvae of the Japanese pest insect *Spodoptera eridania* (Boisduval).

RESULTS AND DISCUSSION

The diethyl ether extract of the root bark contained a variety of limonoids which were detected by the characteristic colour with Ehrlich's reagent on TLC. A powder, insoluble in 50% hexane–diethyl ether, from the extract of the air-dried root bark was fractionated using silica flash chromatography with methanol–methylene dichloride and hexane–diethyl ether solvent systems, and semipreparative normal and reverse phase HPLC. Two new limonoids, named salannal (**1**) and meliacarpinin E (**2**), and four known congeners (**3–6**) were obtained as white noncrystalline solids. Of these compounds, salannal (**1**) was especially sensitive to traces of acid, and gradually decomposed on a silica column and during NMR studies in $CDCl_3$. Salannin (**3**), deacetylsalannin (**4**), nimbolinin B (**5**) and nimbolidin B (**6**) were identified by comparing their NMR data with the reported information.

Salannal (**1**) was assigned the molecular formula $C_{34}H_{44}O_{10}$ from the $[M+1]^+$ ion at m/z 613 in the FAB-mass spectrum and from the ^1H NMR spectra, which, at 27° and 45°, showed the presence of three tertiary and one olefinic methyls, a carbomethoxy and a formyl group, and a 3-furyl moiety along with a tigloyl, an acetyl and a hydroxy group. A comparison of the data with those of compounds **3–5** and sendanal (**7**) [8], isolated from the Japanese plant, and decoupling and NOE experiments suggested that **1** was a salannin type ring C-seco limonoid with a 4α-formyl group, and

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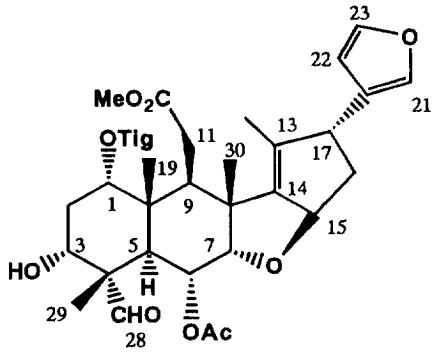
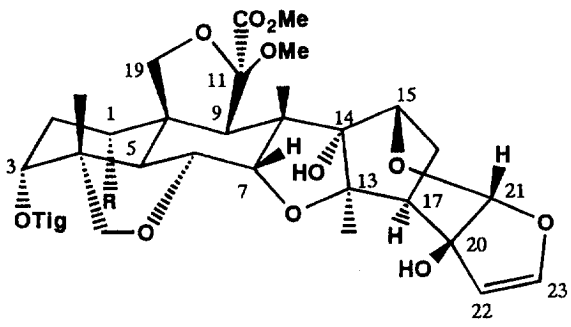


1



2 R = OH
8 R = OAc

allowed us to deduce the structure **1**. Some pertinent points related to the structural study are as follows.

The substitution pattern around the A-ring, namely, that **1** has 1α-tigloyloxy and 3α-hydroxyl groups, was deduced from a long range coupling between H-1β at δ 5.07 (*t*, J = 2.9 Hz) and H-3β at δ 3.75 (*dt*, J = 8.8 and 2.6 Hz) and an abnormal high field shift of an 11-CO_2Me signal at δ 3.23 (*cf* δ 3.24 and 3.20 in **3** and **4** and δ 3.35 in 1α-acetoxysalannin [8]) due to shielding by the tigloyl group. The significant depression of H-5 (δ 3.63) in **1** compared to δ 2.82 and 2.73 in **3** and **4** is attributed to the anisotropic effect of a 4α-formyl group. The depression of H-5 and the formyl proton (δ 9.73) relative to those in sendanal (**7**) (δ 3.05 and 9.50) can be attributed to the effect of hydrogen bonding between the 3α-OH and 28-formyl carbonyl group. A long range coupling of the formyl proton with the 4β-Me group and NOEs between the 4-Me and H-3β and 6β (δ 5.26) also supports the presence of the 4α-CHO group. The α-orientation of H-15, which shows a homoallylic coupling with 13-Me, is clarified by its coupling (*t*, J = 7.6 Hz) with H_2-16, the same with as salannin (**3**).

Meliacarpinin E (**2**), $C_{33}H_{44}O_{13}$ (HRFAB-mass spectrum *m/z*: 671.2698 $[M+Na]^+$; δ +1.8 mmu), showed the presence of hydroxyl (3600–3300 cm^{-1}), ester (1740 cm^{-1}) and conjugated ester (1705 cm^{-1}) groups in the IR spectrum. The ^1H NMR spectrum was almost superimposable on that of meliacarpinin C (**8**) [1] from the same source, particularly with respect to the chemical shifts of protons attached to C-7, C-15 to C-18 and C-21 to C-23 including the 11-OMe, 29-Me and 30-Me and two OH groups at C-14 and C-20, except for the lack of one acetyl group. These data suggested that **2** differed from **8** only in the A-ring.

The presence of a 1α-OH group was deduced from the fact that the H-9 signal was observed at a down field of δ 3.73 due to the effect of the 1α-hydroxyl group in a 1,3-diaxial relationship. The substitution pattern around the A-ring, including the stereochemistry and the presence of C-19/C-11 and C-28/C-6α ether linkages, was clearly elucidated by NOE observations between the 4β-Me (δ 0.97) signal and the H-3β, 6β (δ 3.95), 19α (δ 4.13) and 28β (δ 3.53) signals. Other NOE connectivities similar to those in **8** also supported the structure **2** and its stereochemistry.

The antifeedant activities of the isolated limonoids **2–6** against the third-instar larvae of the Japanese pest insect *Spodoptera eridania* (Boisduval) were tested by a conventional leaf disk method [7]. Meliacarpinin E (**2**) showed the most potent activity at 50 ppm, corresponding to a concentration of 1 μg cm^{-2}, similar to those of meliacarpinins A–D. Other C-seco limonoids **3–6** showed only week activities, **3–5**: 1000 ppm and **6**: 500 ppm. Compound **1** was not tested because it had decomposed during spectral studies before the test.

EXPERIMENTAL

^1H and ^{13}C NMR: with 400 and 100 MHz in $CDCl_3$. FTIR: in $CHCl_3$. $[\alpha]_D$, UV and CD: in MeOH.

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

Extraction and isolation. The dried bark (530 g) was extracted with Et_2O (20 l.), 15°, 2 weeks, to yield 6.0 g of an extract, which was flash chromatographed on silica gel with 0.5–10% MeOH–CH_2Cl_2, and the limonoid fractions eluted wtih 1.0% MeOH–CH_2Cl_2 were rechromatographed on a flash column with 50–0% hexane–Et_2O. Each resulting limonoid fr. was sepd through HPLC on μPorasil and μBondasphere semi-prep 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 along with 6 trichilins, 3 azedarachins and 4 meliacarpinins previously reported: **1** (0.7 mg), **2** (0.6 mg), **3** (0, 8 mg), **4** (3, 5 mg), **5** (1.0 mg) and **6** (0.8 mg).

Salannal (**1**), $C_{34}H_{44}O_{10}$: FAB-MS *m/z*: 613 $[M+1]^+$; $[\alpha]_D^{20}$ +67° (*c* 0.05).

Meliacarpinin E (**2**), $C_{33}H_{44}O_{13}$: HRFAB-MS *m/z*: 671.2698 $[M+Na]^+$, Δ + 1.8 mmu; $[\alpha]_D^{20}$ −10° (*c* 0.05). UV λ_{max} nm (ε): 213 (10 000): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 − 3300, 1740, 1705, 1655 and 1625. CD nm: $\Delta\varepsilon_{230}$ +9.8 and $\Delta\varepsilon_{215}$ −36.

Bioassay of the antifeedant. The antifeedant potential

Table 1. ^{1}H NMR data of compounds **1** and **2** (400 MHz, $CDCl_3$)

H	1	2	H	1	2
1	5.07 *t* (2.9)	3.69 *dt* (8.1, 3.0)	21	7.25 *m*	5.63 *s*
2α	2.21 *dt* (1.65, 2.7)	2.21 *m*	22	6.29 *s* (*br*)	4.88 *d* (2.9)
2β	2.03 *dt* (16.5, 2.8)	2.10 *m*	23	7.33 *t* (1.7)	6.38 *d* (2.9)
3	3.75 *dt* (8.8, 2.6)	5.10 *t* (2.8)	28α	9.73 *s*	3.48 *d* (*br*) (7.7)
5	3.68 *d* (12.1)	2.94 *d* (12.8)	28β		3.53 *d* (7.7)
6	5.26 *dd* (12.1, 2.9)	3.95 *dd* (12.8, 2.8)	29 (Me)	1.08 *s*	0.97 *s*
7	4.03 *d* (2.6)	4.52 *d* (2.9)	30 (Me)	1.41 *s*	1.54 *s*
9	2.84 *dd* (8.4, 3.0)	3.73 *s*	Ac	1.98 *s*	—
11	2.25 *dd* (15.7, 3.3)	—	OH(1α, 3α)	2.72 *d* (8.8)	2.58 *d* (8.1)
	2.32 *dd* (15.7, 8.4)	—	(14α)	—	4.22 *s*
15	5.48 *dd* (*br*) (7.8, 6.4)	4.13 *s* (*br*)	(20β)	—	6.17 *s*
16α	2.10 *dd* (*br*) (12.6, 7.8)	2.18 *ddd* (13.2, 6.2, 2.6)	OMe	—	3.39 *s*
16β	2.25 *dd* (*br*) (12.6, 6.4)	1.86 *dd* (13.2, 1.3)	CO_2Me	3.23 *s*	3.83 *s*
17	3.63 *d* (*br*) (7.8)	2.14 *dd* (6.2, 1.3)	Tig		
18 (Me)	1.65 *d* (*br*) (1.5)	1.52 *s*	3'	6.94 *qq* (7.1, 1.5)	6.86 *qq* (7.5, 1.6)
19	0.97 *s*	3.79 *d* (9.1)	4' (Me)	1.86 *dq* (7.1, 1.0)	1.83 *dq* (7.5, 1.1)
		4.13 *d* (9.2)	5' (Me)	1.94 *dq* (1.5, 1.0)	1.84 *dq* (1.6, 1.1)

of the isolated compounds was measured by a conventional leaf disk method [7] against the third-instar larvae of *S. eridania*. Five disks of Chinese cabbage treated with the sample were arranged with another 5 control disks immersed in Me_2CO alone in a Petri dish, 10 larvae were placed in the centre, and the score for the treated and untreated leaves eaten by the larvae in 2–24 hr was evaluated at appropriate intervals. From these choice tests at 50, 100, 150, 200, 300, 400, 500 and 1000 ppm concentrations, the minimum inhibitory concentration of each limonoid was determined.

Acknowledgements—We would like to thank Dr H. Naoki (Suntory Institute for Bioorganic Research) and Mr N. Nakayama (Nippon Roche Research Center), respectively, for SI-MS and FAB-MS measurements. We are also grateful to Mr K. Takezaki (Kagoshima Prefectural Agricultural Experiment Station) for the supply of the insects.

REFERENCES

1. Nakatani, M., Huang, R. C., Okamura, H., Iwagawa, T., Tadera, K. and Naoki H. (1995) *Tetrahedron* **51**, 11731.
2. Huang, R. C., Okamura, H., Iwagawa, T., Tadera, K. and Nakatani, M. (1995) *Phytochemistry* **38**, 593.
3. Huang, R. C., Zhou, J-Bo, Suenaga, H., Takezaki, K., Tadera, K. and Nakatani, M. (1995) *Biosci. Biotech. Biochem.* **59**, 1755.
4. Henderson, R., McCrindle, R., Melera, A. and Overton, K. H. (1968) *Tetrahedron* **24**, 1525.
5. Kubo, I., Matsumoto, A. and Matsumoto, T. (1986) *Tetrahedron* **42**, 489.
6. Kraus, W. and Bokel, M. (1981) *Chem. Ber.* **114**, 267.
7. Wada, K. and Munakata, K. (1976) *J. Agr. Food Chem.* **16**, 471.
8. Ochi, M., Kotsuki, H. and Tokoroyama, T. (1978) *Chem. Letters* 621.