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AZEDARACHIN C, A LIMONOID ANTIFEEDANT FROM *MELIA AZEDARACH*

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Key Word Index—*Melia azedarach*; Meliaceae; insect antifeedant; azedarachin; limonoid.

Abstract—A new limonoid, azedarachin C, was isolated as an insect antifeedant from the root bark of Chinese *Melia azedarach* and the structure was elucidated by spectroscopic means.

INTRODUCTION

Recently, we isolated a new meliacarpinin [1], six trichilins [2] and three azedarachins [3], azedarachin A, 12-*O*-acetylazedarachin A and 12-*O*-acetylazedarachin B, as insect antifeedants from the root bark of the Chinese Meliaceae plant *Melia azedarach* L. In the continuing study of limonoid antifeedants from the plant, we have isolated a new limonoid, named azedarachin C, as an antifeedant against the larvae of the voracious pest insect *Spodoptera exigua* Hübner (Boisduval). Azedarachin C has no oxygen-function at C-12 and thus is different from known azedarachins.

RESULTS AND DISCUSSION

The antifeeding limonoids from *M. azedarach* were very sensitive to a trace of acid and gradually decomposed on a silica column. Therefore, flash chromatography and HPLC separation techniques were used for the isolation. Azedarachin C (**1**) was isolated by very careful combined use of normal- and reversed phase HPLC of an active fraction from the flash chromatography of a powder, insoluble in 50% hexane–ether, from the ether extract of the root bark.

Azedarachin C (**1**) was assigned the molecular formula $C_{32}H_{42}O_{10}$ from the $[M + 1]^+$ ion at m/z 587 in the SI-mass spectrum and from the ^1H NMR data (Table 1). Taking into acount the circular dichroism (CD) data ($\Delta\varepsilon_{301} - 19$; $n - \pi^*$ of 11-keto group) and IR data (3458 cm^{-1}; OH, 1736 cm^{-1}; ester and 1703 cm^{-1}; CO), these facts allowed us to predict **1** to be 12-dehydroxyazedarachin B. The ^1H NMR spectrum was very similar to that of 12-*O*-acetylazedarachin B (**2**), including the signals due to a 2-methyl-propanoyl group, except for the lack of one acetoxyl group and some changes of chemical shifts (Table 1). The 12-methylene protons were observed as a singlet at δ2.47 similar to those (both δ2.46*s*) of trichilin D [4] and meliatoxin A_2 [5].

Compound **1** has a free 1α-hydroxyl and a 3α-acetoxyl group and this substitution pattern around the A-ring is the same as in **2** and azedarachin A (**3**), as was shown by the fact that the H-9 signal in **1** was at δ4.56 due to the

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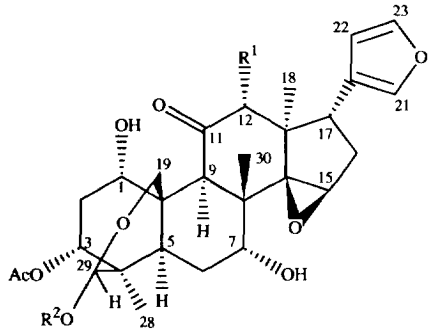

	R^1	R^2
1	H	COCH Me_2
2	OAc	COCH Me_2
3	OH	COCH Me CH_2 Me
4	OAc	COCH Me CH_2 Me

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

H	1		2		H	1		2	
1	4.11 *m*		4.27 *m*		18	1.24 *s*		1.32 *s*	
2α	1.88 *d(br)*	(16.4)	1.89 *d(br)*	(16.4)	19a	4.38 *d*	(13.0)	4.28 *d*	(12.4)
2β	2.87 *dt*	(16.6, 4.4)	2.82 *dt*	(16.4, 4.6)	19b	4.41 *d*	(13.0)	4.34 *d*	(12.4)
3	5.34 *d(br)*	(3.9)	5.31 *d(br)*	(4.4)	21	7.14 *m*		7.13 *d(br)*	(1.4)
5	2.63 *dd*	(13.8, 4.0)	2.72 *dd*	(13.9, 4.0)	22	6.14 *m*		6.15 *m*	
6α	1.73 *dt*	(14.3, 3.7)	1.73 *dt*	(14.3, 3.7)	23	7.37 *t*	(1.4)	7.33 *t*	(1.6)
6β	2.07 *dt*	(2.2, 14.3)	2.04 *dt*	(2.1, 14.3)	28	0.83 *s*		0.83 *s*	
7	3.69 *m*		3.67 *m*		29	5.81 *s*		5.80 *s*	
9	4.56 *s*		4.61 *s*		30	1.10 *s*		1.17 *s*	
12	2.47 *s*		5.28 *s*		2′	2.62 *hept*	(6.9)	2.61 *hept*	(7.0)
15	3.70 *s*		3.75 *s*		3′	1.19 *d*	(7.0)	1.19 *d*	(7.0)
16α	2.27 *ddd*	(13.5, 6.2, 0.9)	2.25 *dd*	(13.2, 6.3)		1.20 *d*	(7.0)	1.20 *d*	(7.0)
16β	1.88 *dd*	(13.5, 11.0)	1.92 *dd*	(13.2, 11.1)	Ac	2.11 *s*		1.98 *s*	
17	2.76 *dd*	(11.0, 0.69)	2.98 *dd*	(11.1, 6.1)				2.11 *s*	

effect of the 1-hydroxyl in a 1,3-diaxial relationship. On the other hand, the *S*(exo)-configuration at C-29 was assigned from the chemical shift of the 3β-H, which was observed at the low position of δ5.34, as found in sendanin [6], and all of the trichilins and azedarachins, when compared to endo-compounds (29*R*-benzoate: δ5.07 [7]). This assignment was also supported by an NOE observation between the 28-Me and H-29 signals. Finally, the stereochemistry of **1** (Fig. 1) was elucidated by NOE enhancements between the 18-Me and the H-9, H-21 and H-22 signals, the 30-Me and the H-7β and H_b-19 signals, and long range couplings between the H-9 and 30-Me and the H-5 and H_a-19 signals, respectively.

Azedarachin C (**1**) at 400 ppm (corresponding to a concentration of *ca* 8 μg cm^{-2}) inhibited the feeding of the larvae of the Japanese pest insect *Spodoptera exigua* Hübner (Boisduval) when arranged by the conventional leaf disk method [8].

EXPERIMENTAL

^{1}H NMR: 400 MHz in $CDCl_3$. $[\alpha]_D$, UV and CD: in MeOH. IR: in $CHCl_3$. Bioassay of the antifeedant was done by the leaf disk method with the larvae of *S. exigua*.

Plant material. The root bark was collected in October 1992 at Guangzhou, 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 1–10% MeOH–CH_2Cl_2, and the limonoid frs eluted with 1–1.5% MeOH–CH_2Cl_2 were rechromatographed on a flash column with 20% hexane–Et_2O. Each limonoid fr. was sepd through HPLC using normal, μ-Porasil and reversed-phase columns, μ-Bondapac C_{18}, with 0.5–2% MeOH–CH_2Cl_2 and 25–40% H_2O–MeOH as the solvents, respectively, to give **1** (0.5 mg) and 3 known limonoids **2** (2.5 mg), **3** (1 mg) and **4** (0.7 mg).

Azedarachin C (**1**). $C_{32}H_{42}O_{10}$; SIMS *m/z*: 587 [M + 1]$^+$; $[\alpha]_D^{22}$ − 41° (*c* 0.08); UV λ_{max}^{nm} (ε): 207 (4200); CD nm; $\Delta\varepsilon_{225}$ + 2.2, $\Delta\varepsilon_{243}$ − 3.3 and $\Delta\varepsilon_{301}$ − 19; IR ν_{max}: cm^{-1}: 3458, 1736 and 1703.

12-O-*Acetylazedarachin* B (**2**). $C_{34}H_{44}O_{12}$; SIMS *m/z* 645[M + 1]$^+$; $[\alpha]_D^{22}$ + 55° (*c* 0.13); UV λ_{max}^{nm} (ε): 213(3000); CD nm: $\Delta\varepsilon_{217}$ + 25 and $\Delta\varepsilon_{308}$ − 10. Azedarachin A (**3**). $C_{33}H_{44}O_{11}$; SIMS *m/z* 617 [M + 1]$^+$; $[\alpha]_D^{22}$ − 10° (*c* 0.05); UV λ_{max}^{nm} (ε): 213(4300); CD nm: $\Delta\varepsilon_{223}$ + 6 and $\Delta\varepsilon_{310}$ − 26. 12-*O*-Acetylazedarachin A (**4**). $C_{35}H_{46}O_{12}$; SIMS *m/z* 659[M + 1]$^+$; $[\alpha]_D^{22}$ + 7.5° (*c* 0.08); UV λ_{max}^{nm} (ε): 212 (6300); CD nm: $\Delta\varepsilon_{218}$ + 17 and $\Delta\varepsilon_{309}$ − 14.

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