Two Novel Azadirachtin Derivatives from *Azadirachta indica*

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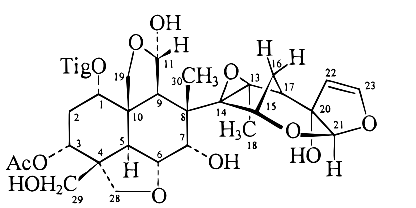
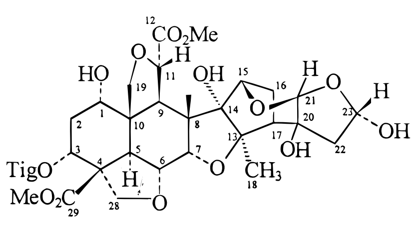
Two novel compounds, the first 29-oxymethylene azadirachtin analogue, 29-oxymethylene-11- demethoxycarbonyl-11α-hydroxyazadirachtin (azadirachtin M) (**1**) and 22,23-dihydro-23α-hydroxy-3-tigloyl-11-deoxyazadirachtinin (azadirachtin N) (**2**), together with known compound 11-*epi*-azadirachtin H were isolated from a methanolic extract of the seed kernels of *Azadirachta indica*. The structures of **1** and **2** were elucidated on the basis of spectral methods.

During the past two decades, the biological activity and chemical constituents of *Azadirachta indica* A. Juss. (Meliaceae) (neem tree) have been investigated intensively in both developed and developing countries. Many plant parts of *Azadirachta indica* display an array of effects on insects, including as an ovipositor-deterrent, an antifeedant, and other inhibitory activities.[1,2] More than 100 compounds have been isolated from the various parts of the neem tree, and several reviews have been published to date.[2−5] Most of the active principles belong to the group of tetranortriterpenoids, especially the azadirachtin analogues.

In the present investigation on the constituents of neem tree seed kernels, three azadirachtin derivatives were isolated and characterized: two of them are new compounds, named azadirachtins M (**1**) and N (**2**). Their structures were elucidated as 29-oxymethylene-11-demethoxycarbonyl-11α-hydroxyazadirachtin and 22,23-dihydro-23α-hydroxy-3-tigloyl-11-deoxyazadirachtinin, respectively. In addition, a known compound, 11-*epi*-azadirachtin H,[6] was also obtained.



1



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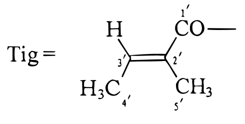

Compound **1**, from its negative-ion HRFABMS at *m*/*z* 633.2518 $[M - H]^+$, together with the ^{13}C NMR and DEPT spectral data, indicated a molecular formula of $C_{32}H_{42}O_{13}$. The ^1H NMR and ^{13}C NMR spectra of **1** were similar to those of other compounds in the azadirachtin group,[7] especially 11-*epi*-azadirachtin H and azadirachtin I,[6,8] and the characteristic H-11 signal at δ 6.10 (s) (pyridine-d_5) showed compound **1** to be a 11-demethoxycarbonyl azadirachtin derivative. Further comparison of the ^{13}C NMR and ^1H NMR spectra of **1** with those of 11-*epi*-azadirachtin H revealed that the signals at δ 174.7 ($-CO_2-$), 52.3 (OCH_3) in the ^{13}C NMR spectrum and δ 3.68 (3H, s) in the ^1H NMR spectrum of 11-*epi*-azadirachtin H were absent in compound **1**. In addition, signals were observed in the ^{13}C NMR spectrum at δ 61.2 (CH_2) and in the ^1H NMR spectrum at δ 4.12 (1H, d) and 3.86 (1H, d), consistent with a CH_2OH group at C-29. The assumption was confirmed from the HMBC spectrum, in which long-range couplings were observed for C-29 [δ 61.2 (CH_2)] to H-28a [δ 4.71 (1H, d)] and for C-3 [δ 68.8 (CH)], C-4 [δ 49.1 (quaternary carbon)], C-5 [δ 35.1 (CH)], and C-28 [δ 72.1 (CH_2)] to H-29 [δ 4.12 (1H, d), 3.86 (1H, d)]. Unlike the ^1H NMR spectrum of azadirachtin H, the H-11 ^1H NMR signal of **1** occurred at δ 6.10 as a broad singlet, suggesting a very small or undetectable coupling between H-11 and H-19. A molecular model indicating a small coupling between these two protons assumed H-11 to be in the β configuration. This was further supported by the ROESY spectrum of **1**. The stereochemistry at the other chiral centers in **1** was identical to that of azadirachtin, as supported by its ^1H NMR, $^1H-^1H$ COSY, and ROESY spectra. Thus, compound **1** was determined structurally as 29-oxymethylene-11-demethoxycarbonyl-11α-hydroxyazadirachtin.

Compound **1** is a novel biodegradation product of azadirachtin, and belongs to neither the azadirachtin group (where C-29 is a methoxycarbonyl group) nor the meliacarpin group (where C-29 is a methyl group). It is the first compound where C-29 is an oxymethylene group that has been isolated from *A. indica*. The degree of oxidation at C-29 in **1** is between those of azadirachtin H and azadirachtin I,[8] so azadirachtin M could be considered as an intermediate of the biosynthesis between the meliacarpin group and the azadirachtin group.

Compound **2**, from its negative-ion HRFABMS at *m*/*z* 679.2630 $[M - H]^+$, together with the ^{13}C NMR and DEPT spectra, possessed a molecular formula of $C_{33}H_{44}O_{15}$. The ^{13}C NMR spectrum of **2** was similar to that of 1-tigloyl-3-acetyl-11-methoxyazadirachtinin.[7] The characteristic ^{13}C NMR signals at about δ 93 (quaternary carbon) and δ 95 (quaternary carbon) suggested the opening of the C-13, C-14 epoxy ring [at δ 68 (quaternary carbon) and δ 70

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Table 1. ^{13}C NMR Spectral Data for Compounds **1** and **2** (100 MHz)[a]

carbon	1	2
C-1	74.3 d	69.4 d
C-2	28.6 t	32.8 t
C-3	68.8 d	68.3 d
C-4	49.1 s	53.3 s
C-5	35.1d	34.3 d
C-6	75.0 d	72.5 d
C-7	74.0 d	80.9 d
C-8	45.2 s	48.7 s
C-9	48.9 d	47.5 d
C-10	49.1 s	51.4 s
C-11	101.7 d	77.5 d
C-12		175.5 s
C-13	69.4 s	92.3 s
C-14	71.2 s	95.0 s
C-15	76.5 d	80.3 d
C-16	27.0 t	28.4 t
C-17	49.3 d	53.3 d
C-18	19.7 q	25.4 q
C-19	71.7 t	70.6 t
C-20	83.2 s	83.0 s
C-21	109.1 d	106.7 d
C-22	109.5 d	49.4 t
C-23	146.7 d	96.4 d
C-28	72.1 t	73.0 t
C-29	61.2 t	172.7 s
C-30	21.5 q	15.5 q
OAc	20.8 q	
	170.2 s	
$COOCH_3$		53.3 q
		52.5 q
tigloyl		
C-1′	166.7 s	166.1 s
C-2′	129.4 s	127.8 s
C-3′	137.7 d	139.4 d
C-4′	14.2 q	14.6 q
C-5′	12.1 q	12.1 q

[a] Compound **1** was measured in pyridine-d_5, compound **2** in $CDCl_3$; chemical shifts are in ppm, with TMS as internal standard.

Table 2. ^1H NMR Spectral Data for Compounds **1** and **2** (400 MHz)[a]

proton	1	2
H-1	6.02 (dd, 2.8, 2.8)	3.61 (m)
H-2α	2.77 (dt, 11.6, 2.5)	2.11 (m)
H-2β	2.44 (dt, 2.5, 3.2)	2.06 (m)
H-3	5.85 (dd, 2.6, 2.6)	5.63 (dd, 2.6, 2.5)
H-5	4.06 (d, 6.8)	3.07 (d, 12.6)
H-6	4.53 (dd, 12.6, 2.1)	4.32 (dd, 12.8, 3.0)
H-7	5.09 (br, s)	4.65 (d, 2.8)
H-9	3.55 (s)	3.27 (br, s)
H-11	6.10 (br, s)	4.62 (s)
H-15	4.78 (br, s)	4.16 (s)
H-16a	1.88 (m)	2.18 (m)
H-16b	1.38 (d, 5.6)	1.98 (m)
H-17	2.57 (d, 5.6)	2.08 (m)
H-18	2.49 (s)	1.52 (s)
H-19a	4.16 (d, 9.2)	3.54 (d, 12.8)
H-19b	4.26 (d, 9.2)	3.91 (d, 13.2)
H-21	6.56 (s)	5.50 (s)
H-22	5.28 (d, 2.8)	2.10 (m), 2.30 (m)
H-23	6.59 (d, 2.0)	5.48 (dd, 11.4, 5.2)
H-28a	4.71 (d, 7.8)	3.50 (d, 8.9)
H-28b	4.10 (d, 7.8)	3.92 (d, 8.9)
H-29a	4.12 (d, 11.0)	
H-29b	3.86 (d, 10.8)	
H-30	1.49 (s)	1.37 (s)
CH_3COO	1.76 (s)	OCH_3-12 3.68 (s)
$COOCH_3$		OCH_3-29 3.76 (s)
tigloyl		
H-3′	7.37 (qq, 7.0, 1.5)	6.85 (qq, 9.4, 1.6)
H-4′	1.65 (d, 7.0)	1.80 (d, 7.4)
H-5′	1.90 (s)	1.81 (s)
OH-7	6.58 (s)	*b*
OH-11	7.95 (br, s)	
OH-20	7.12 (s)	
OH-29	6.81 (br, s)	

[a]Compound **1** was measured in pyridine-d_5 and compound **2** in $CDCl_3$. (Chemical shifts are in ppm and coupling constants in Hz, with TMS as internal standard.) [b] The OH proton signals were not observed in compound **2**.

(quaternary carbon)] and the formation of a C-7, C-13 ether bridge. Accordingly, its skeleton could be proposed as the same as that of azadirachtinin.[7] The signals at δ 107.3 (C-11, quaternary carbon), 145.8 (C-23, CH) and 108.2 (C-22, quaternary carbon) in the ^{13}C NMR spectrum of 1-tigloyl-3-acetyl-11-methoxy azadirachtinin[7] were absent in that of **2**, with the double bond between C-22 and C-23 replaced by signals at δ 96.4 (CH) and 49.4 (CH_2) suggesting that C-23(δ 96.4) is a hemiacetal carbon. These inferences were also supported by the $^1H-^1H$ COSY, $^{13}C-^1H$ COSY, and COLOC NMR spectra obtained for **2**. Long-range couplings were observed for C-21 [δ 106.7 (CH)] to H-23 (δ 5.48, 1H, dd) and for C-23 [δ 96.4 (CH)] to H-22 (δ 2.10, 2.30, each 1H, m). The configuration of the OH-23 group was determined from the NOESY spectrum of **2**, with NOE interaction observed between H-23 and H-30 (3H, s) and H-23 and H-7. Thus, OH-23 was determined as having an α substitution. The NOESY spectrum showed a NOE interaction between H-11 and H-30, so H-11 was assigned as β. Therefore, compound **2** was elucidated as 22,23-dihydro-23α-hydroxy-3-tigloyl-11-deoxyazadirachtinin.

11-*epi*-Azadirachtin H was identified by comparison of its IR and UV data with the reported values,[6] as well as by a detailed analysis of the ^1H and ^{13}C NMR spectral data.

Experimental Section

General Experimental Procedures. All the mps were obtained on a Kofler apparatus and uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter in MeOH solution. UV spectra were measured with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^1H NMR, ^{13}C NMR, and 2D-NMR spectra were recorded on Bruker AM-400 MHz and a DRX-500 spectrometers with TMS as internal standard. MS data were recorded on a VG Autospec-3000 spectrometer.

Plant Material. Seeds of *A. indica* were collected in Mandalay, Myanmar in August 1994, where the plant is cultivated. The plant material was identified by Prof. Tianlu Ming, Kunming Institute of Botany, Acedemia Sinica, Kunming, Yunnan, People's Republic of China, where the specimen was deposited.

Extraction and Isolation. The dehulled and air-dried neem seed kernels (1.3 kg) were extracted with petroleum ether three times at room temperature, then the defatted kernels were extracted with methanol six times at room temperature. The combined extracts were evaporated *in vacuo*. The residue was suspended in H_2O, and then extracted with petroleum ether, EtOAc, and *n*-BuOH, respectively. The EtOAc and *n*-BuOH layers were concentrated *in vacuo* to give 32 and 45 g of residues, respectively. The EtOAc extract was repeatedly chromatographed over silica gel. The column was eluted with $CHCl_3-Me_2CO$ (9:1−3:1) to give 30 fractions. Fractions 8−22 were purified on reversed-phase C_{18} silica gel columns using CH_3OH-H_2O (3:2) as eluent to yield **2** (24 mg) and 11-*epi*-azadirachtin H (8 mg). The *n*-BuOH extract was fractionated on D-101, eluted with CH_3OH-H_2O with increasing CH_3OH content. The fraction eluted with 70% CH_3OH was chromatographed on a silica gel column by elution with $CHCl_3-CH_3OH$ (4:1−2:1) and then on a reversed-phase C_{18} silica gel column by elution with CH_3OH-H_2O (1:1) to yield **1** (12 mg).

Azadirachtin M (1): colorless needles (MeOH); mp >350 °C; $[\alpha]^{24}_D$ −20.0° (*c* 0.60, CH_3OH); UV (MeOH) λ_{max} (log ε)

210 (3.98) nm; IR (KBr) ν_{max} 3434, 2935, 1719, 1650, 1622, 1272, 1046 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 616 [M − H_2O]$^+$ (5), 598 (20), 533 (22), 516 (10), 265 (15), 151 (100), 95 (48), 83 (75); HRFABMS *m*/*z* 633.2518 [M − 1]$^+$ (calcd for $C_{32}H_{41}O_{13}$, 633.2547).

Azadirachtin N (2): white powder (MeOH); mp 155−157 °C; $[\alpha]^{23}_{D}$ +12.1° (*c* 1.30, CH_3OH); UV (MeOH) λ_{max} (log ϵ) 217 (3.89) nm; IR (KBr) ν_{max} 3407, 2956, 1729, 1650, 1383, 1038 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 680 [M]$^+$ (0.5), 662 (2), 621 (2), 564 (4), 522 (57), 449 (8), 273 (10), 83 (100); HRFABMS *m*/*z* 679.2630 [M − 1]$^+$ (calcd for $C_{33}H_{43}O_{15}$, 679.2602).

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