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11-EPI-AZADIRACHTIN D: AN EPIMERIC AZADIRACHTIN ANALOGUE FROM *AZADIRACHTA INDICA*

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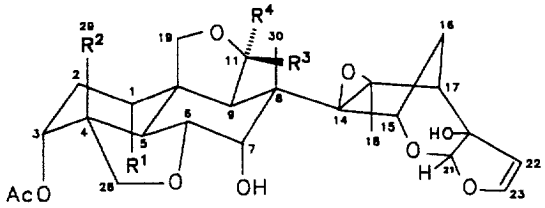
Key Word Index—*Azadirachta indica*; Meliaceae; seeds; tetranortriterpenes; 11-epi-azadirachtin D.

Abstract—A new tetranortriterpene, 11-epi-azadirachtin D (1-tigloyl-3-acetyl-11α-hydroxy-4β-methyl-meliacarpin) has been isolated from the methanolic extracts of *Azadirachta indica* seeds. Its structure is proposed on the basis of various spectral analyses. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

It has been known for a long time that plants have evolved elaborate defence mechanisms to counter insect predation. The tetranortriterpene azadirachtin (**1**) [1, 2] and its analogues [3–8], the constituents of *Azadirachta indica*, have remarkable insect anti-feedant and ecdysis inhibiting properties. The orientation of the hydroxyl group at C-11 is β in all the known analogues of azadirachtin except azadirachtin H (**2**) [9, 10]. We report here the isolation of 11-epi-azadirachtin D (**4**) having a novel α-oriented 11-hydroxyl group.



(1)	R^1=Tig	R^2=CO_2Me	R^3=OH	R^4=CO_2Me
(2)	R^1=Tig	R^2=CO_2Me	R^3=H	R^4=OH
(3)	R^1=Tig	R^2=Me	R^3=OH	R^4=CO_2Me
(4)	R^1=Tig	R^2=Me	R^3=CO_2Me	R^4=OH

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of **4** was similar to the spectrum of azadirachtin D (**3**) [11, 12] in the following signals: (i) the two olefinic protons at H-22, H-23 (δ 5.04 and 6.44) of the dihydrofuran ring and a low field singlet for H-21 at δ 5.68; (ii) the four spin system H-15, H-16α,β, H-17 (δ 4.66, 1.68, 1.32 and 2.39); (iii) the four spin system H-1, H-2α,β, H-3 (δ 4.91, 2.23, 2.08 and 4.68); (iv) the AB system of H-28α,β, and H-19α,β at δ 3.82 and 4.21; (v) the three spin system H-5, H-6, H-7 (δ 3.10, 4.15 and 4.71); (vi) the three methyl signals H-18, H-29 and H-30 (δ 2.06, 1.74 and 1.05).

The proton connectivities were established by the ^{1}H-^{1}H COSY spectra and the multiplicities of all the carbon atoms were assigned by SEFT experiments. The large upfield shift of the C-30 methyl by 0.68 ppm and a similar shift of C-6 and C-29 methyl as compared to that of **3** [11, 12] (δ 4.69, H-6; δ 1.94, H-29; δ 1.73, H-30, Table 1) indicated that the carbomethoxyl group at C-11 was β-oriented, resulting in an anisotropic shielding. This was confirmed by NOESY experiments, wherein an NOE was observed between COOMe at C-11 and the β-oriented H-30 as well as H-6 protons, indicating the *R*-configuration at C-11. The stereochemistry at all the other centres were identical to those of **3** as indicated by the ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY and NOESY spectra. Based on various spectral analyses, the compound **4** was characterized as 11-epi-azadirachtin D (1-tigloyl-3-acetyl-11α-hydroxy-4β-methylmeliacarpin). The OH(11)—O(13) distance in the MM2 minimized structure (Alchemy molecular model program) was found to be 4.2 A° indicating the absence of intramolecular hydrogen bonding, as in the case of 11-epi-azadirachtin H (**2**). This would result in a greater degree of free rotation about the C-8 C-14 bond unlike azadirachtin A (**1**). Earlier reports on some azadirachtin analogues [13] indicate that free rotation

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Table 1. ^{1}H NMR and ^{13}C NMR data for compounds **3** and **4***

Position	3		4	
	δ_C	δ_H	δ_C	δ_H
1	70.90	4.91 (*dd*, 2.9, 2.4)	70.9	4.91 (*br s*)
2	27.99	2.30 (*ddd*, 16.6, 2.9, 2.4)	28.0	2.23 (*br d*, 16.7)
		2.13 (*ddd*, 16.6, 2.9, 2.4)		2.08 (*br d*, 16.7)
3	70.32	5.15 (*dd*, 2.9, 2.4)	70.3	4.68 (*br s*)
4	42.44	—	42.4	—
5	35.47	3.16 (*d*, 12.7)	35.5	3.10 (*d*, 12.7)
6	72.81	4.69 (*dd*, 12.7, 2.0)	72.7	4.15 (*dd*, 12.7, 2.7)
7	74.87	4.71 (*d*, 2.0)	74.9	4.71 (*br s*)
8	45.08	—	45.0	—
9	44.71	3.34 (*s*)	44.7	3.33 (*s*)
10	49.96	—	49.9	—
11	103.99	—	104.0	—
12	171.76	—	171.7	—
13	68.50	—	69.0	—
14	69.90	—	70.0	—
15	76.38	4.67 (*d*, 3.4)	76.3	4.66 (*d*, 3.7)
16	24.97	1.70 (*ddd*, 13.0, 3.4, 5.4)	25.0	1.68 (*m*)
		1.33 (*d*, 13.0)		1.32 (*d*, 13.1)
17	48.98	2.34 (*d*, 5.4)	48.9	2.39 (*d*, 5.1)
18	18.42	2.05 (*s*)	18.3	2.06 (*s*)
19	69.97	3.85 (*d*, 9.3)	70.0	3.82 (*d*, 9.3)
		4.21 (*d*, 9.3)		4.21 (*d*, 9.3)
20	83.62	—	83.6	—
21	108.72	5.71 (*s*)	108.8	5.68 (*s*)
22	107.33	5.03 (*d*, 2.9)	107.3	5.04 (*d*, 2.9)
23	146.82	6.44 (*d*, 2.9)	146.9	6.44 (*d*, 2.9)
28	76.88	4.16 (*d*, 10.3)	76.7	3.82 (*d*, 9.3)
		3.78 (*d*, 10.3)		4.21 (*d*, 9.3)
29	18.95	1.94 (*s*)	19.0	1.74 (*s*)
30	21.14	1.73 (*s*)	21.1	1.05 (*s*)
COOMe	53.20	3.68 (*s*)	53.2	3.69 (*s*)
OAc	20.88	1.94 (*s*)	20.9	1.92 (*s*)
OAc(C=O)	170.09	—	170.0	—
7-OH		2.80 (*s*)		2.78 (*s*)
11-OH		5.00 (*s*)		5.05 (*s*)
20-OH		3.24 (*s*)		3.14 (*s*)
1′(C=O)	166.34	—	166.3	—
2′(C=C)	128.56	—	128.6	—
3′(C=C)	137.59	6.92 (*qq*, 7.3, 1.5)	137.4	6.88 (*br q*, 7.1)
4′	14.34	1.78 (*dq*, 7.3, 1.0)	14.3	1.77 (*br d*, 6.7)
5′	11.96	1.85 (*dq*, 1.5, 1.0)	11.9	1.85 (*br s*)

* In $CDCl_3$, at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

about the C-8 and C-14 bond results in enhanced biological activity.

EXPERIMENTAL

Neem seeds were collected in Tamil Nadu, India, during December 1994. The crushed neem seed kernels were first extracted with hexane to remove the oil completely. The residue was then extracted with MeOH. The MeOH extract was then partitioned between aq. MeOH and EtOAc. The EtOAc extract was subjected to gross fractionation using a flash column to remove highly polar materials. Azadirachtin containing fractions were then subjected to repeated CC over silica gel (100–200 mesh) with $CHCl_3$–MeCN as the solvent system and semi-prep HPLC using a μ-Bondapac C-18 column (eluting with MeCN–H_2O, 30:70), resulting in the isolation of pure compound **4**.

Compound 4. Microcrystalline solid, mp 170–174°; $[\alpha]_D^{27}$ −1.27° (*c*, 0.55, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 293 (3.57); IR ν_{max}^{CHCl3} cm^{-1}; 3440 (OH), 1740 (Ester), 1610 (C=C). HRLSIMS *m/z*: 677.278475 $[MH]^+$ (calcd for $C_{34}H_{44}O_{14}$ 677.276462), 659 $\{[MH]^+ - H_2O\}^+$, 641 $\{659 - H_2O\}^+$. ^{1}H NMR and ^{13}C NMR: Table 1.

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