

1-Tigloyl-3-acetylazadirachtol, a New Limonoid from the Marrango Tree, *Azadirachta excelsa* Jack (Meliaceae)^Ψ

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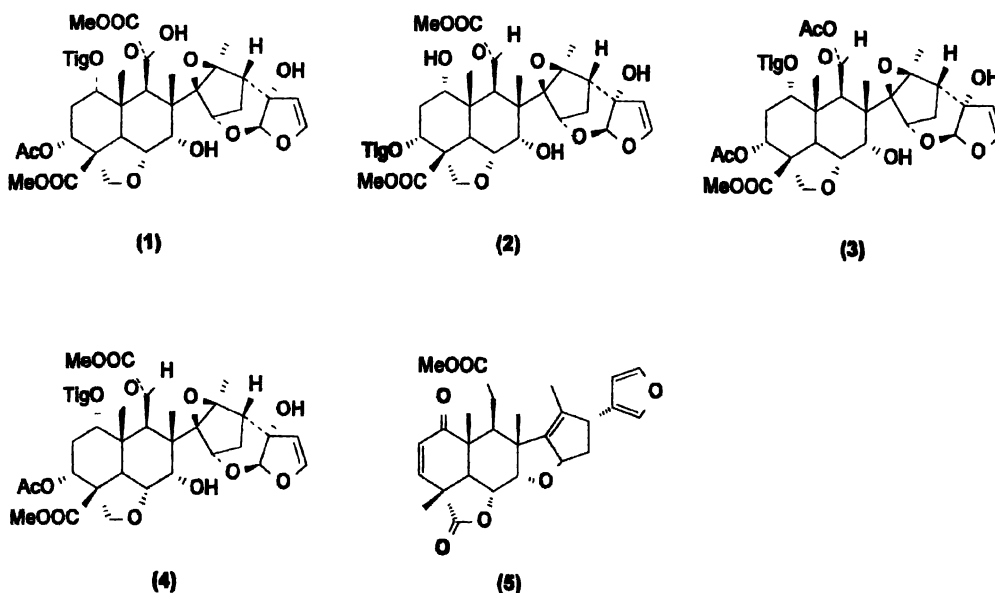
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The structure of a new limonoid from the Marrango tree, *Azadirachta excelsa* Jack, has been established as 1-tigloyl-acetylazadirachtol (4) by 1D and 2D ¹H and ¹³C nmr spectra.

The constituents of *A. indica* A. Juss (Meliaceae), also known as 'neem tree', have been investigated intensively by different research groups during the past decades because of their effects on feeding behaviour and development of insects and other biological activities². Up till now, azadirachtin^{2–9} is the most interesting component present in neem seed extracts since it has been found to be not only a very potent insect antifeedant but also an insect growth regulating agent². Recently, another *Azadirachta* species occurring in the Philippines and other South Asian countries, *A. excelsa* Jack (Meliaceae), the 'marrango tree', was found to be a promising source for natural products with considerable insect feeding or development controlling properties^{10,11}. We report on the isolation and structure determination by nmr of a new compound, 1-tigloyl-3-acetylazadirachtol (4) from *A. excelsa*.

ether and aqueous methanol, and 3-tigloylazadirachtol (2)¹² and nimbolide (5)¹³ by extraction of *A. excelsa* bark with ethyl acetate. MPLC over silica gel (Si60, 45–63 μm 300 × 40 mm, gradient system PE/EtOAc/MeOH) and HPLC (LiChrospher RP-18, 5 μm 250 × 4.6 mm, MeCN/water = 3.5/6.5) led to the pure compounds.

The structure of 4 was established by ¹H and ¹³C nmr spectroscopy (Tables 1 and 2). The assignments of the ¹H nmr signals were achieved by means of H,H-COSY and nuclear Overhauser difference spectroscopy (n.O.e., Table 3). The ¹³C signals were assigned on the basis of DEPT spectra and by comparison to already described azadirachtin analogs. Comparison of the ¹³C-spectroscopic data of 1^{4,7} and 4 showed that the hemiacetal carbon signal of C-11 is shifted from δ 104.10 in 1 to δ 79.19 in 4 (Table 2) As proved by D₂O exchange, only one tertiary OH (20-OH, δ



Results and Discussion

Azadirachtin (1), marrangin (3)¹¹ and 1-tigloyl-3-acetylazadirachtol (4) were isolated by extraction of *A. excelsa* seeds with petrol ether, followed by partition between petrol

2.78) and one secondary OH group (7-OH, δ 3.01) are present in the spectrum of 4. This led us to assume that 4 is an azadirachtin-11-desoxo compound of the tigloylazadirachtol type. In the H,H-COSY and long-range H,H-

^ΨDedicated to Professor T. R. Govindachari on the occasion of his 80th Birth Anniversary.

TABLE 1—¹H Nmr DATA (250 MHz; CDCl₃, TMS = 0) OF COMPOUNDS 1-4

¹ H	1	2	3	4
1-H	4.75 dd (2.9, 3.1)	3.52 ddd (2.3, 2.7, 5.8)	5.30 t (2.8)	4.71 dd (2.9, 2.9)
2-H _α	2.34 dd (16.7, 2.9, 2.7)	2.32 ddd (16.3, 2.3, 2.8)	2.34 m	2.29 dd (2.9, 2.9)
2-H _β	2.13 ddd (16.7, 3.1, 2.9)	2.22 ddd (16.3, 2.7, 2.8)	2.34 m	2.99 dd (2.9, 2.9)
3-H	5.50 dd (2.9, 2.7)	5.53 dd (2.8, 2.8)	5.54 t (2.9)	5.50 dd (2.9, 2.9)
5-H	3.35 d (12.5)	3.33 d (12.7)	3.33 d (12.5)	3.39 d (12.3)
6-H	4.60 dd (12.5, 2.7)	4.55 dd (12.7, 2.6)	4.48 dd (12.5, 2.7)	4.53 dd (12.5, 2.8)
7-H	4.75 d (2.7)	4.72 d (2.5)	4.71 d (2.6)	4.77 d (2.8)
9-H	3.34 s	3.19 d (1.3)	2.81 s	3.28 d
11-H	—	4.47 d (1.3)	6.16 s	4.59 s (br)
15-H	4.67 d (3.4)	4.58 d (3.9)	4.58 d (3.7)	4.63 d (3.6)
16-H _α	1.73 ddd (13.0, 5.1, 3.4)	1.33 d (12.9)	1.67 ddd (13.4, 5.3, 3.9)	1.67 ddd (13.0)
16-H _β	1.31 d (13.0)	1.65 ddd (12.9, 3.9, 5.3)	1.29 d (13.4)	1.27 d (13.0)
17-H	2.38 d (5.1)	2.36 d (5.3)	2.34 m	2.37 d (5.3)
18-H	2.01 s	2.04 s	1.83 s	1.98 s
19-H _α	3.63 d (9.6)	3.49 d (9.4)	3.75 d (9.8)	3.70 d (9.8)
19-H _β	4.15 d (9.6)	3.95 d (9.4)	3.93 d (9.8)	3.88 d (9.8)
21-H	5.65 s	5.66 s	5.67 s	5.67 s
22-H	5.05 d (2.9)	5.03 d (2.9)	5.06 d (2.9)	5.06 d (2.9)
23-H	6.46 d (2.9)	6.43 d (2.9)	6.47 d (2.9)	6.45 d (2.9)
28-H _α	4.08 d (9.0)	4.04 d (9.0)	4.12 d (8.9)	4.09 d (8.9)
28-H _β	3.76 d (9.0)	3.83 d (9.0)	3.78 d (8.9)	3.71 d (8.9)
30-H	1.74 s	1.45 s	1.39 s	1.42 s
1-OH	—	3.41 dd (5.8)	—	—
7-OH	3.02 s	3.29 s (br)	2.70 s	3.01 s
11-OH	5.05 s	—	—	—
20-OH	3.11 s	2.78 s (br)	2.71 s	2.78 s (br)
12-OCH ₃	3.68 s	3.76 s	—	3.76 s
29-OCH ₃	3.76 s	3.76 s	3.80 s	3.80 s
3-OAc	1.95 s	—	1.97 s	1.96
11-OAc	—	—	2.00 s	—
Tigloyl-				
3'-H	6.93 qq (7.0, 1.5)	6.95 qq (7.0, 1.3)	6.92 qq (7.1, 1.4)	6.92 qq (7.0, 1.5)
4'-H	1.78 dq (7.0, 1.1)	1.79 dq (7.0, 1.3)	1.78 dq (7.1, 1.1)	1.78 dq (7.0, 1.3)
5'-H	1.85 dq (1.5, 1.1)	1.84 dq (1.3, 1.3)	1.85 s (br)	1.85 dq (1.5, 1.3)

 TABLE 2—¹³C Nmr DATA (62.89 MHz; CDCl₃, TMS = 0) OF COMPOUNDS 1-4

¹³ C	1	2	3	4	C-18	18.49 q	18.56 q	18.93 q	16.10 q
C-1	70.51 d	69.37 d	71.23 d	70.26 d	C-19	69.07 t	71.43 t	71.12 t	69.50 t
C-2	29.37 t	32.09 t	30.64 t	30.20 t	C-20	83.55 s	83.71 s	83.57 s	83.60 s
C-3	66.99 d	67.69 d	67.12 d	66.99 d	C-21	108.70 d	109.16 d	108.76 d	108.68 d
C-4	52.52 s	53.34 s	52.47 s	52.50 s	C-22	107.30 d	107.53 d	107.53 d	107.52 d
C-5	37.06 d	35.18 d	36.90 d	36.97 d	C-23	147.00 d	146.79 d	147.15 d	147.14 d
C-6	73.79 d	74.32 d	74.03 d	76.49 d	C-30	173.20 s	174.12 s	173.26 s	173.31 s
C-7	74.37 d	73.68 d	72.99 d	73.73 d	12-OCH ₃	21.33 q	21.35 q	20.54 q	21.03 q
C-8	45.41 s	44.04 s	43.26 s	44.39 s	29-OCH ₃	53.52 q	53.01 q	—	52.72 q
C-9	44.69 d	43.80 d	47.10 d	44.97 d	CH ₃ COO	52.72 q	52.63 q	52.72 q	52.72 q
C-10	50.19 s	51.24 s	47.40 s	49.26 s	CH ₃ COO	169.50 s	—	169.71 s	169.72 s
C-11	104.10 s	79.48 d	99.64 d	79.19 d	CH ₃ COO	20.88 q	—	20.96 q	20.91 q
C-12	171.70 s	173.52 s	—	172.58 s	CH ₃ COO	—	—	170.00 s	—
C-13	68.53 s	66.59 s	66.51 s	66.79 s	C-1'	—	—	20.54 q	—
C-14	69.69 s	69.43 s	69.36 s	69.50 s	C-2'	166.22 s	167.08 s	165.87 s	166.14 s
C-15	76.43 d	76.16 d	76.18 d	76.17 d	C-3'	128.60 s	128.49 s	128.77 s	128.65 s
C-16	25.04 t	25.08 t	25.14 t	25.22 t	C-4'	137.50 d	138.86 d	137.44 d	137.52 d
C-17	48.67 d	48.99 d	48.28 d	48.39 d	C-5'	14.29 q	14.69 q	14.27 q	14.33 q
						11.94 q	12.09 q	11.96 q	11.97 q

Table 2-(contd.)

COSY 2D-nmr spectra of **4**, a crosspeak was observed between 9-H δ 3.28 and the signal at δ 4.59. Irradiation at δ 4.59 caused a n.O.e. on 9-H and 30-H (Table 3). These effects are in accordance with a proton attached to C-11. These n.O.e. effects, H,H-COSY couplings and a similar chemical shift of C-11 (δ 79.48) are also observed in the spectrum of 3-tigloylazadirachtol (**2**)¹². All other n.O.e.'s (Table 3) are typical for azadirachtin and related compounds, particularly the n.O.e. 3'-H/18-H indicating that the tiglate group is attached to C-1^{4,7}.

TABLE 3—NUCLEAR OVERHAUSER EFFECTS IN THE ¹H NMR SPECTRUM (250 MHz, CDCl₃) OF 1-TIGLOYL-3-ACETYLAZADIRACHTOL (**4**)

Irradiated	Observed
18-H	9-H, 17-H, 3'-H
30-H	6-H, 7-H; 15-H, 19-H _{β} ; 11-H; 21-H
9-H	18-H; 5-H, 11-H
11-H	9-H, 30-H
3'-H	18-H, 4'-H

Bioassay :

In the 24h dual choice antifeedant test against the Mexican bean beetle *E. varivestis*¹⁴, compounds **1–5** showed high activity (Table 4), marrangin (**3**) and 1-tigloyl-3-acetylazadirachtol (**4**) being more potent than azadirachtin (**1**).

TABLE 4—ANTIFEEDANT ACTIVITY AGAINST *E. varivestis*

[ppm]	1	2	3	4	5
EC ₅₀	13	30	6	6	90
EC ₁₀₀	120	150	50	50	>500

Compounds **1–5** were also tested for antimicrobial activity¹⁵ against *Bacillus subtilis* (Gram-positive) and *Pseudomonas stutzeri* (Gram-negative) (Table 5). Nimbolide (**5**) showed inhibitory effects even at a concentration as low as 0.5 μ g/spot. All other compounds possessed no bactericidal activity up to a concentration of 100 μ g/spot.

TABLE 5—BACTERICIDAL ACTIVITY AGAINST *Bacillus subtilis* AND *Pseudomonas stutzeri*

[μ g/spot]	1	2	3	4	5
<i>B. subtilis</i>	>100	>100	>100	>100	5
<i>P. stutzeri</i>	>100	>100	>100	>100	5

Experimental

A. excelsa, bark and seed kernels, were kindly provided by Prof. Dr. H. Schmutterer and Dr. K. Ermel, Institut für Phytopathologie, Universität Giessen.

Nmr measurements were performed with a Bruker WM 250 instrument : ¹H nmr 250 MHz, ¹³C nmr 62.89 MHz; solvent CDCl₃, internal standard TMS. Mass spectra (ELMS, 70 eV) were recorded on a Varian MAT 311 A or Varian

MAT 44 S instrument. Optical rotation was recorded on a Perkin-Elmer 241 polarimeter.

Isolation of 1, 3, and 4 : Ground *A. excelsa* seed kernel (140 g) was stirred with petrol ether (5 \times 3 dm³; b.p.30–50°) for 24 h. Partition between petrol ether and aqueous methanol of the extract gave 45 g oil and 1.5 g methanol/water phase. Chromatography of the methanol/water phase on LiChroprep Si 60 (45–63, μ m 300 \times 40 mm gradient system PE/EtOAc/MeOH) gave 10 fractions.

Azadirachtin (1) : HPLC on LiChrospher (RP-18, 5 μ m, 250 \times 4.6 mm, MeCN/water = 3.5/6.5; flow 0.8 ml min⁻¹, λ 216 nm and RI detection) of fraction 8 (120 mg) gave 1 mg **1** (t_R = 16 min), R_f = 0.18 (TLC, silica gel 60 F₂₅₄ 0.25 mm plates, Merck, Darmstadt, PE/EtOAc/acetone/EtOH = 72 : 13 : 12 : 3).

Marrangin (3) : HPLC on LiChrospher (RP-18, 5 μ m, 250 \times 4.6 mm, MeCN/water = 3.5 : 6.5; flow 1 ml min⁻¹, λ 216 nm and RI detection) of fraction 10 (90 mg) gave 1.5 mg **3** (t_R = 11 min), R_f = 0.10 (Si60, PE/EtOAc/acetone/EtOH = 72 : 13 : 12 : 3).

1-Acetyl-3-tigloylazadirachtol (4) : HPLC on LiChrospher (RP-18, 5 μ m, 250 \times 4.6 mm, MeOH/water = 4:6; flow 1 ml min⁻¹, λ 216 nm and RI detection) of fraction 9 (80 mg) gave 5.5 mg **4** (t_R = 14 min), R_f = 0.18 (Si60, PE/EtOAc/acetone/EtOH = 72 : 13 : 12 : 3); C₃₅H₄₄O₁₅; calcd. 704.2667, found m/z 704.2675 (HRMS); ¹H and ¹³C nmr spectra in Tables 1 and 2.

λ (nm)	589	578	546	436	405	365	313
$[\alpha]_D^{25}$	-12	-16	-20	-51	-72	-103	-26

(CHCl₃, 0.1)

Isolation of 2 and 5 : Powdered *A. excelsa* bark (500 g) was successively stirred three times with 5 dm³ petrol ether, ethyl acetate, and methanol for 24 h. Chromatography on LiChroprep Si60 (45–63 μ m, 300 \times 40 mm, gradient system PE/EtOAc/MeOH) of the crude EtOAc extract (4.5 g) gave 9 fractions.

3-Tigloylazadirachtol (2) : HPLC on LiChrospher (RP-18, 5 μ m, 250 \times 16 mm, MeCN/water = 4 : 6; flow 4.5 ml min⁻¹, λ 216 nm and RI detection) of fraction 8 (200 mg) gave 2.5 mg **2** (t_R = 14 min), R_f = 0.16 (Si60, PE/EtOAc/acetone/EtOH = 72 : 13 : 12 : 3).

Nimbolide (5) : HPLC on LiChrospher (RP-18, 5 μ m, 250 \times 16 mm, MeCN/water = 4 : 6; flow 4.5 ml min⁻¹, λ 216 nm and RI detection) of fraction 3 (340 mg) gave 33 mg **5** (t_R = 20 min), R_f = 0.33 (Si60, PE/EtOAc/acetone/EtOH = 72 : 13 : 12 : 3).

Acknowledgement

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