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STRUCTURE DETERMINATION BY NMR OF AZADIRACHTIN AND RELATED COMPOUNDS FROM *AZADIRACHTA INDICA* A. JUSS (MELIACEAE)

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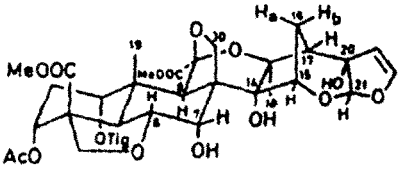
Abstract: *Two novel limonoids 3-deacetyl-3-cinnamoylazadirachtin (6) and 1-tigloyl-3-acetyl-11-methoxyazadirachtinin (8) have been isolated from Azadirachta indica extracts in addition to azadirachtin (4), 22,23-dihydro-23ß-methoxyazadirachtin (5) and 3-tigloylazadirachtol (7). The assignment of the structures (5) - (8) and the reassignment of the azadirachtin structure as (4) was achieved on the basis of detailed ^{1}H and ^{13}C n.m.r. spectroscopic analyses.*

INTRODUCTION

The constituents of *Azadirachta indica* A. Juss (Meliaceae), also known as "neem tree", have been investigated intensively by different groups during the past two decades because some of them have considerable insect feeding or development controlling properties.[1] Up to now azadirachtin[2] 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 agent.[1] Structure formula (1) was published in 1975[3] and has been accepted for 10 years. Some doubt remained, however, in certain parts of the structure which were not consistent with all n.m.r. data. In 1985 two alternative structures (2)[4a] and (3)[5] were discussed which, however, were still not completely consistent with all n.m.r. data. During our own work on biologically active principles of the neem tree and *Melia azedarach* L. we have isolated several compounds related very closely to azadirachtin[4] which prompted us to start a reinvestigation of the n.m.r. spectra of azadirachtin itself. In the following we report on the isolation and structure determination by n.m.r. of the new compounds 3-deacetyl-3-cinnamoylazadirachtin (6),[4c] 1-tigloyl-3-acetyl-11-methoxyazadirachtinin (8),[6] 22,23-dihydroxy-23ß-methoxyazadirachtin (5),[6,7] and 3-tigloylazadirachtol (7),[4a,6,8] and on the unambigous structure proof of azadirachtin as (4).[6,7] Assignments of the ^{1}H n.m.r. signals were achieved using homonuclear decoupling, and measurements of the nuclear Overhauser effects (n.O.e.) in the FT difference spectra. The ^{13}C signals were assigned on the basis of the two dimensional $^{1}H,^{13}C$ heteroscalar correlated spectra,[9] H,C COLOC experiments,[10] and the DEPT spectra.[11]

†Deceased August, 7, 1986.



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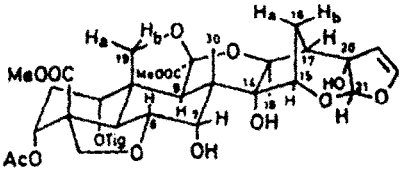

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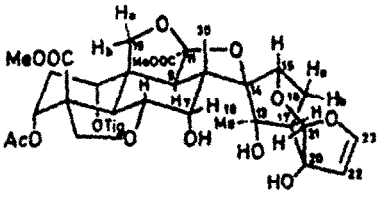

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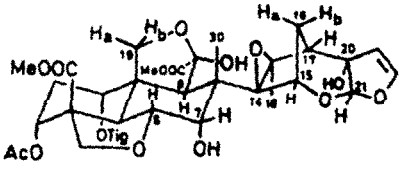

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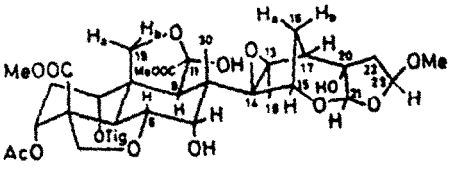

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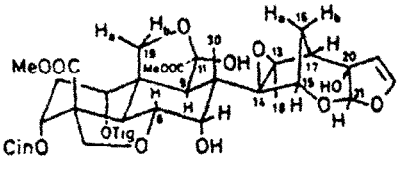

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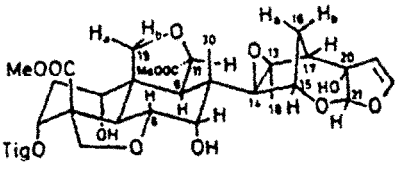

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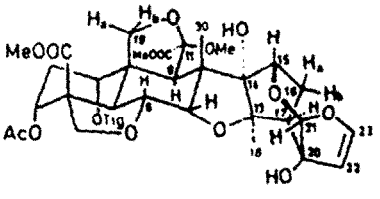

(8)

RESULTS

Isolation of Compounds (4) - (8)

Azadirachtin (4), 22,23-dihydro-23B-methoxyazadirachtin (5), and 3-tigloylazadirachtol (7) were isolated by extraction of neem seeds with acetone followed by partition between petrol ether and aquous methanol, and subsequent chromatography of the methanolic phase.[12] 3-Deacetyl-3-cinnamoylazadirachtin (6) was isolated chromatographically from neem leaf ether extracts, and 1-tigloyl-3-acetyl-11-methoxyazadirachtinin (8) from neem bark ether extracts.

Table 1. 250 MHz ^{1}H n.m.r. data ($CDCl_3$, TMS = 0) of compounds (4) - (8).

	(4)		(5)	(6)	(7)	(8)
1-H	4.75 (dd,2.9;3.1)		4.73 (dd,2.6;2.6)	4.79 (t, 3.0)	3.52 (ddd,2.3;2.7;5.8)[a]	4.81 (dd,2.9;3.1)
2-H$_\alpha$	2.34 (ddd,16.7;2.9;2.7)		2.31 (ddd,16.5;2.6;2.7)	2.46 (dt,16.5;3.0)	2.32 (ddd,16.3;2.3;2.8)	2.28 (ddd,16.7;2.9;2.4)
2-H$_\beta$	2.13 (ddd,16.7;3.1;2.9)		2.22 (ddd,16.5;2.6;2.9)	2.29 (dt,16.5;3.0)	2.06 (dddd,16.3;2.7;2.8;1.0)	2.13 (ddd,16.7;3.1;3.3)
3-H	5.50 (dd,2.7;2.9)		5.49 (dd,2.7;2.9)	5.59 (t,3.0)	5.53 (dd,2.8;2.8)	5.48 (dd,2.4;3.3)
5-H	3.35 (d,12.5)		3.29 (d,12.4)	3.43 (d,12.5)	3.33 (d,12.7)	3.16 (d,12.7)
6-H	4.60 (dd,12.5;2.7)		4.57 (dd,12.4;2.5)	4.63 (dd,12.5;2.7)	4.55 (dd,12.7;2.6)	4.39 (dd,12.7;3.2)
7-H	4.75 (d,2.7)		4.65 (d,2.5)	4.68 (d,2.7)	4.72 (d,2.6)	4.53 (d,3.2)
9-H	3.34 (s)		3.30 (s)	3.38 (s)	3.19 (d,1.3)	3.56 (s)
11-H	-		-	-	4.47 (d,1.3)	-
15-H	4.67 (d,3.4)		4.67 (d,3.4)	4.75 (d,3.3)	4.58 (d,3.9)	4.13 (m)[a]
16-H$_a$	1.73 (ddd,13.0;3.4;5.1)		1.64 (ddd,13.1;3.4;5.2)	1.71 (ddd,13.0;3.3;5.2)	1.65 (ddd,12.9;3.9;5.3)	2.15 (m)[b]
16-H$_b$	1.31 (d,13.0)		1.92 (d,13.1)	1.30 (d,13.0)	1.33 (d,12.9)	1.85 (m)[c]
17-H	2.38 (d,5.1)		2.47 (d,5.2)	2.38 (d,5.2)	2.36 (d,5.3)	2.12 (m)[d]
18-H	2.01 (s)		2.00 (s)	2.03 (s)	2.04 (s)	1.50 (s)
19-H$_a$	3.63 (d,9.6)		3.63 (d,9.7)	3.66 (d,9.6)	3.49 (d,9.4)[b]	3.73 (d,9.7)
19-H$_b$	4.15 (d,9.6)		4.15 (d,9.7)	4.18 (d,9.6)	3.95 (d,9.4)	4.21 (d,9.7)
21-H	5.65 (s)		5.51 (s)	5.66 (s)	5.66 (s)	5.64 (s)
22-H	5.05 (d,2.9)	(α)	2.38 (dd,14.7;6.7)	5.05 (d,2.9)	5.03 (d,2.9)	4.88 (d,2.9)
		(β)	2.22 (dd,14.7;3.2)			
23-H	6.46 (d,2.9)		5.18 (dd,6.7;3.2)	6.45 (d,2.9)	6.43 (d,2.9)	6.39 (d,2.9)
28-H$_{\alpha,\beta}$	4.08 (d,9.0)		4.06 (d,8.9)	4.11 (d,9)	3.83 (d,9.0)	3.66 (d,8.8)
	3.76 (d,9.0)		3.74 (d,8.9)	3.87 (d,9)	4.04 (d,9.0)	4.03 (d,8.8)
29-H	-		-	-	-	-
30-H	1.74 (s)		1.76 (s)	1.76 (s)	1.45 (s)	1.57 (s)
1-OH	-		-	-	3.41 (dd,5.8;1.0)	-
3-OH	-		-	-	-	-
7-OH	2.89(br.s)		2.79(br.s)	2.89 (s)	3.29(br.s)	-
11-OH	5.05(s)		5.02(br.s)	5.07 (s)	-	-
14-OH	-		-	-	-	4.33 (s)
20-OH	2.92(br.s)		2.97(br.s)	2.96 (s)	2.78(br.s)	6.07 (s)
11-OCH_3	-		-	-	-	3.37 (s)
12-OCH_3	3.68 (s)		3.67 (s)	3.68 (s)	3.76 (s)	3.72 (s)
23-OCH_3	-		3.42 (s)	-	-	-
29-OCH_3	3.76 (s)		3.79 (s)	3.82 (s)	3.76 (s)	3.77 (s)
CH_3COO	1.95 (s)		1.95 (s)	-	-	1.99 (s)
Tigloyl						
3'-H	6.93 (qq,7.0;1.5)		6.89 (qq,7.0;1.5)	6.93 (qq,7.0;1.5)	6.95 (qq,7.0;1.3)	6.93 (qq,7.0;1.5)
4'-H	1.78 (dq,7.0;1.1)		1.77 (dq,7.0;1.1)	1.65 (dq,7.0;1.5)	1.79 (dq,7.0;1.3)	1.81 (dq,7.0;1.1)
5'-H	1.85 (dq,1.5;1.1)		1.85 (dq,1.5;1.1)	1.80 (dq,1.5;1.5)	1.84 (dq,1.3;1.3)	1.85 (dq,1.1;1.5)
Cinnamoyl						
2'/6'-H	-		-	7.48-7.36 (m)	-	-
3'/5'-H	-		-		-	-
4'-H	-		-		-	-
7'-H	-		-	7.65 (d,16)	-	-
8'-H	-		-	6.26 (d,16)	-	-

[a] in C_6D_6: 3.68
[b] in C_6D_6: 3.44

[a] in C_6D_6: 4.14 (m)
[b] in C_6D_6: 2.16 (ddd,13.0; 6.0;2.5)
[c] in C_6D_6: 1.91 (br.s)
[d] in C_6D_6: 2.03 (br.d,6.0)

Table 2. 62.89 MHz ^{13}C n.m.r. data [$CDCl_3$ and $(CD_3)_2SO/D_2O$, TMS = 0] of compounds (4) - (8).

	(4)		(5)		(6)	(7)	(8)	
	$CDCl_3$	$(CD_3)_2SO/D_2O$	$CDCl_3$	$(CD_3)_2SO/D_2O$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$(CD_3)_2SO/D_2O$
C-1	70.51 d	70.88	70.39 d	70.88	71.20 d	69.37 d	70.26 d	70.07
C-2	29.37 t	28.68	29.74 t	28.83	29.98 t	32.09 t	30.15 t	29.25
C-3	66.99 d	66.91	66.94 d	66.95	67.72 d	67.69 d	66.97 d	66.59
C-4	52.52 s	51.96	52.43 s	52.52	52.94 s	53.34 s	52.87 s	52.02
C-5	37.06 d	36.10	37.19 d	36.64	37.55 d	35.18 d	36.34 d	35.83
C-6	73.79 d	73.90	73.82 d	74.16	74.27 d	74.32 d	71.93 d	71.36
C-7	74.37 d	73.82 73.71	74.19 d	73.71 73.60	76.75 d	73.68 d	82.70 d	82.28
C-8	45.41 s	45.89	45.07 s	46.02	45.18 s	44.04 s	51.46 s	50.86
C-9	44.69 d	44.52	44.69 d	44.59	45.85 d	43.80 d	47.92 d	47.16
C-10	50.19 s	50.24	50.05 s	50.16	50.60 s	51.24 s	49.78 s	49.05
C-11	104.10 s	104.07 103.97	104.23 s	104.12 104.22	104.54 s	79.48 d	107.25 s	106.58
C-12	171.70 s	171.36	171.68 s	171.49	172.25 s	173.52 s	170.14 s	169.10
C-13	68.53 s	67.93	68.38 s	67.91	69.45 s	66.59 s	95.10 s	94.23
C-14	69.69 s	70.39	69.34 s	69.42	70.64 s	69.43 s	93.26 s	92.61 92.50
C-15	76.43 d	75.60	77.05 d	77.11	74.74 d	76.16 d	81.42 d	82.27
C-16	25.06 t	25.72	24.47 t	25.79	25.63 t	25.08 t	29.63 t	29.09
C-17	48.67 d	47.47	49.95 d	52.00	49.02 d	48.99 d	50.81 d	50.01
C-18	18.49 q	18.16	18.39 q	18.07	18.71 q	18.56 q	26.67 q	26.23
C-19	69.07 t	68.54	69.09 t	68.47	69.36 t	71.43 t	70.44 t	69.09
C-20	83.55 s	81.78 81.69	80.96 s	80.24 80.33	83.76 s	83.71 s	86.44 s	85.93 85.82
C-21	108.70 d	108.73	106.87 d	107.14	109.03 d	109.16 d	109.41 d	108.61
C-22	107.30 d	107.13	48.10 t	48.68	107.99 d	107.53 d	108.24 d	108.40 108.45
C-23	147.00 d	145.96	105.75 d	105.73	147.17 d	146.79 d		
C-29	173.20 s	173.56	173.25 s	173.69	173.91 s	174.12 s	173.34 s	173.83
C-30	21.33 q	21.46	21.26 q	21.92	21.59 q	21.35 q	17.63 q	17.29
$COOCH_3$	53.52 q 52.72 q	52.38 52.05	52.22 q 52.77 q	54.34 52.48	53.25 q 52.76 q	53.01 q 52.63 q	52.87 q 53.70 q	52.73 52.66
11-OCH_3	-	-	-	-	-	-	52.49 q	51.88
23-OCH_3	-	-	55.79 q	54.34	-	-	-	-
CH_3COO	169.50 s	169.33	169.57 s	169.85	-	-	169.72 s	169.97
CH_3COO	20.88 q	20.36	20.86 q	20.50	-	-	21.07 q	20.68
Tigloyl								
C-1'	166.22 s	166.22	166.15 s	166.25	166.15 s	167.08 s	166.84 s	166.18
C-2'	128.60 s	128.00	128.67 s	127.90	128.83 s	128.49 s	128.84 s	128.88
C-3'	137.50 d	137.85	137.41 d	138.14	138.29 d	138.86 d	138.18 d	138.47
C-4'	14.29 q	13.96	14.29 q	14.07	14.50 q	14.69 q	14.18 q	13.91
C-5'	11.94 q	11.54	11.94 q	11.58	12.11 q	12.09 q	12.16 q	11.91
Cinnamoyl								
C-1'	-	-	-	-	134.50 s	-	-	-
C-2'/6'	-	-	-	-	128.41 d	-	-	-
C-3'/5'	-	-	-	-	129.30 d	-	-	-
C-4'	-	-	-	-	130.97 d	-	-	-
C-7'	-	-	-	-	146.02 d	-	-	-
C-8'	-	-	-	-	117.63 d	-	-	-
C-9'	-	-	-	-	166.15 s	-	-	-

Structure of Azadirachtin (4)

The results of our reinvestigation of the n.m.r. spectra are listed in tables 1 and 2. Most of the ^1H and ^{13}C n.m.r. data are in accord with the published values.[3] The signals of 7-H and 15-H, however, had to be reassigned on the basis of homodecoupling experiments and n.O.e. measurements in the FT difference spectrum (table 3): Irradiation of the 6-H signal (δ 4.60) converted the doublets at δ 3.35 (5-H) and δ 4.75 into singlets. Saturation of the signal at δ 4.75 gave a n.O.e. on 7-OH and *vice versa*. In $(CD_3)_2SO$ as the solvent a coupling 7-H/7-OH could be observed.[3] In addition the doublet at δ 4.67 couples with 16-H_a. Hence δ 4.75 corresponds to 7-H, δ 4.67 to 15-H. The reassignment of the 7-H and 15-H signals led to a reassignment of the C-7 and C-15 signals (table 2) carried out by $^1H,^{13}C$ heteroscalar correlated 2D spectra:[9] C-7 is now found at δ 74.37 instead of δ 76.43 which corresponds to C-15 and C-6 at δ 73.79. Irradiation of the signal at δ 1.74 (C-19 in structure (1)) gave n.O.e's on 6-H (δ 4.60), 7-H (δ 4.75), 15-H (δ 4.67), the hydroxyl proton (δ 5.05) which was thought to be 14-OH in structure (1), and the two signals at δ 3.63 (negative n.O.e.) and δ 4.15 (positive n.O.e.)[14] corresponding to 30-$H_{a/b}$ in structure (1). Irradiation of 15-H (δ 4.67) gave n.O.e's on 16-$H_{a,b}$ (δ 1.73; 1.31), 21-H (δ 5.65), the low field OH proton supposed to be 14-OH in structure (1), and on the δ 1.74 signal which was also enhanced upon saturation of the OH signal at δ 5.05. The n.O.e's δ 1.74/15-H and δ 1.74/14-OH are not consistent with structure (1). In addition there is no effect on the signals

of 1-H, 2-Hß, and 3-H during irradiation at δ 1.74 which one should expect if this signal would correspond to 19-H. On the basis of these considerations we concluded the oxygen bridge to be located between C-11 and C-19 as depicted in structure formula (2)[4a] which is consistent with most of the n.O.e.'s in question: The δ 1.74 signal corresponds to 30-H, δ 3.63 to 19-H_a, δ 4.15 to 19-H_b. On the basis of the $^1H,^{13}C$ heteroscalar correlated 2D spectra[9] the signals of C-19 and C-30 had to be reassigned as δ 69.07 for C-19, and δ 21.33 for C-30. This assignment was confirmed by the observation of long range coupling C-7/30-H, C-8/30-H, C-10/19-H_a, and C-11/19-H_a (table 4) in a H,C COLOC experiment,[10] optimized for a coupling constant of 10 Hz. The same experiment was used to reassign the quarternary carbon atoms (table 4). Long range coupling was observed for C-11/19-H_b in a H,C COLOC experiment optimized for J = 5 Hz. The α-configuration of the C-1 tigloyloxy group, 9-H, and 13-CH_3 (18-H), was confirmed by n.O.e. enhancements 18-H/9-H (6%), 18-H/3'-H (2%), 9-H/18-H (37%), and 9H/3'H (12%).

Table 3. Nuclear Overhauser effects in the ^1H n.m.r. spectra (250 MHz, $CDCl_3$) of azadirachtin (4) and 22,23-dihydro-23ß-methoxyazadirachtin (5).

	(4)	(5)
Irradiated	observed	observed
7-H	21-H,30-H,7-OH,20-OH	16-H_a,16-H_b,21-H,30-H,7-OH,11-OH,20-OH**)
15-H	16-H_a,16-H_b,21-H,30-H,11-OH	16-H_a,16-H_b,21-H,30-H,7-OH,11-OH,20-OH**)
18-H	9-H,17-H,3'-H,7-OH,20-OH	9-H,17-H,3'-H,7-OH,20-OH
21-H	7-H,7-OH,20-OH	7-H,23-H,7-OH,20-OH
30-H	6-H,7-H,15-H,19-H_a(-),19-H_b(+),11-OH	6-H,7-H,15-H,19-H_a(-),19-H_b(+),11-OH
7-OH	5-H,7-H,21-H	5-H,7-H,21-H
11-OH	9-H,15-H,23-H*),30-H	9-H,30-H
20-OH	21-H	18-H,21-H

*) 11-OH and 22-H cannot be irradiated separately

**) 15-H and 7-H have nearly identical chemical shifts

The ester groups C-12 and C-29 were also assigned using H,C COLOC experiments: Long range coupling was observed for C-12 (δ 171.70) to 12-OCH_3 (δ 3.68) and 9-H, and for C-29 (δ 173.20) to 29-OCH_3 (δ 3.76). Similarly this method was used in order to reassign the carbon signals of C-13, C-15, C-20, and C-22 by long range couplings C-13/15-H, C-13/18-H, C-15/17-H, C-15/21-H, C-20/17-H, C-20/22-H, C-20/23-H, and C-22/23-H (table 4). All these n.m.r. data are consistent with structure formula (2). On the other hand neither structure (1) nor structure (2) can be used in order to explain the strong n.O.e. 21-H/7-H (6%) because the distance between 7-H and 21-H is too big as can be seen from inspections of molecular models of (1) and (2). The n.O.e. 21-H/7-H and the other n.O.e's mentioned above would be consistent with structure formula (3), recently proposed by S.V. Ley et al.[5] However, in no one of structures (1),(2), or (3) a n.O.e. on the low field tertiary hydroxy group [14-OH in (1) and (2), 13-OH in (3)] would be possible upon satura-

Table 4. $^1H,^{13}C$ long range coupling ($CDCl_3$, H,C COLOC experiment, optimized for J=10 Hz) in azadirachtin (4)

δ, ^{13}C signals	δ, ^1H signals
70.51 d (C-1)	2.34 (2-H_α), 3.34 (9-H), 5.50 (3-H)
66.99 d (C-3)	2.34 (2-H_α), 4.75 (1-H), 5.50 (3-H)
52.52 s (C-4)	5.50 (3-H), 4.60 (6-H), 3.35 (5-H)
73.79 d (C-6)	3.35 (5-H), 4.75 (7-H)
74.37 d (C-7)	1.74 (30-H)
45.41 s (C-8)	3.34 (9-H), 1.74 (30-H)
50.19 s (C-10)	2.34 (2-H_α), 3.34 (9-H)
104.10 s (C-11)	3.34 (9-H), 3.63 (19-H_a), 4.15 (19-H_b, optimized for J=5 Hz), 5.05 (11-OH, optimized for J=5 Hz)
171.70 s (C-12)	3.68 (12-OCH_3), 3.34 (9-H)
68.53 s (C-13)	4.67 (15-H), 2.01 (18-H)
69.69 s (C-14)	3.34 (9-H), 1.74 (300-H), 2.01 (18-H, 2.38 (17-H)
76.43 d (C-15)	5.65 (21-H), 2.38 (17-H)
69.07 t (C-19)	3.34 (9-H)
83.55 s (C-20)	5.05 (22-H), 6.46 (23-H), 2.38 (17-H)
107.30 d (C-22)	6.46 (23-H)
173.20 s (C-29)	3.76 (29-OCH_3)

tion of 30-H. Furthermore we did not observe any n.O.e 18/16-H_a,[5] which should be rather strong because these protons are located very closely to each other. With structures (1) - (3) there is no explanation for the chemical shift of C-13 (δ 68.53) and C-14 (δ 69.69) which should appear at lower field if C-13 and C-14 would be connected to tertiary hydroxy or alkoxy groups.[15] In the n.m.r. spectrum of 1-tigloyl-3-acetyl-11-methoxy-azadirachtinin (8), e.g., the C-13 signal is observed at δ 95.10, C-14 at δ 93.26 (*vide infra*).

The n.O.e's on 30-H (6%) and 9-H (8%) on irradiation of the low field tertiary hydroxy group (δ 5.05) and the n.O.e 7-H/21-H led us search for alternative structures. The positions of the tertiary hydroxy groups were determined by deuterium isotope shift experiments in $(CD_3)_2SO$ as the solvent which are frequently used in carbohydrate ^{13}C n.m.r. spectroscopy.[16] Addition of one mole D_2O per hydroxy group to the solution gives a one to one mixture of deuterated and undeuterated species. Under the conditions of slow exchange as it is the case in solvents of high viscosity like DMSO the signals of both isotopomers ROD and ROH can be observed in the ^{13}C n.m.r. spectrum. ß-Isotope shifts were found for C-7 (δ 73.82; 73.71), C-20 (δ 81.78; 81.69) and C-11 (104.07; 103.97) but not for C-13 or C-14 (figure 1) indicating that the tertiary hydroxy group in question is attached to C-11 which in earlier work[3,4a,5] had been assumed to be an acetal carbon.

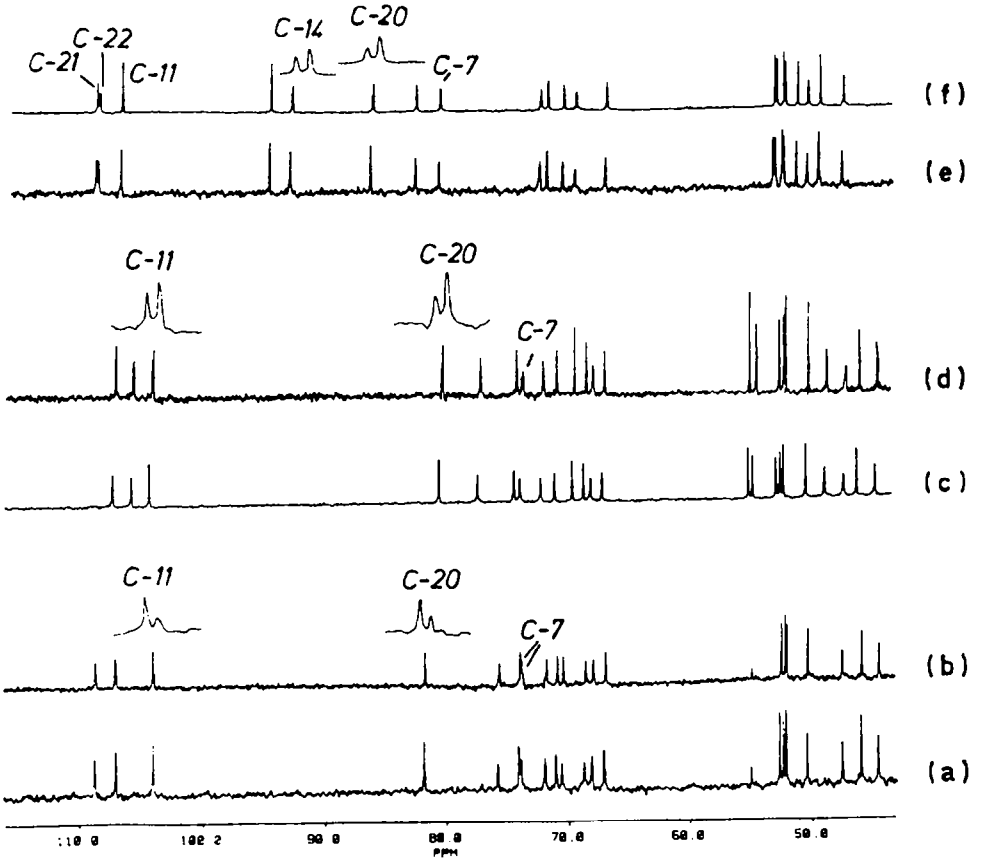

Figure 1. Deuterium Isotope Shifts of Azadirachtin (4), 22,23-Dihydro-23ß-methoxyazadirachtin (5), and 1-Tigloyl-3-acetyl-11-methoxyazadirachtinin (8):

(a) (4) in $(CD_3)_2SO$, (b) (4) in $(CD_3)_2SO/D_2O$; (c) (5) in $(CD_3)_2SO$, (d) (5) in $(CD_3)_2SO/D_2O$; (e) (8) in $(CD_3)_2SO$, (f) (8) in $(CD_3)_2SO/D_2O$.

The chemical shift of C-11 corresponds very well to those found for similar hemiacetal carbons present in certain quassinoids.[17] Consequently C-11 is part of a hemiacetal structure (4), and there is no oxygen bridge connecting C-11 and C-13 [structures (1) and (2)] or C-14 [structure (3)]. The hemiacetal (4) structure was confirmed by a long range coupling C-11/11-OH (δ 5.05) in

to be an epoxide oxygen connected to carbons 13 and 14 on the basis of the chemical shifts of C-13 (δ 68.53) and C-14 (δ 69.69) which are typical for quarternary oxirane carbons.[18] The ß-configuration of the oxiran follows from the strong n.O.e's 7-H/21-H (6%) and 7-OH/21-H (6%) which would not be possible because of a much longer distance between 7-H and 21-H if the oxiran ring would be α-oriented. On the basis of these results we assigned structure (4) to azadirachtin.[6,7] This structure was confirmed by a X-ray diffraction analysis carried out on 1-detigloyl-22,23-dihydroazadirachtin,[19] and an n.m.r. study on azadirachtin-7,11,20-trimethyl ether.[13]

The distinct n.O.e's from ring D on ring B protons and *vice versa* indicate that there is no completely free rotation around the C-8/C-14 bond. This is certainly due to a strong hydrogen bond from 11-OH to the oxiran oxygen determined in the X-ray analysis in addition to the 7-OH/20-OH hydrogen bond.[19] Consequently a doubling of nearly all proton signals was produced by cooling a solution of (4) in THF-d_8 to 183K (table 5).[12] The signals of the two rotamers appear in a ratio of 3:2.

Table 5. Proton n.m.r. data for azadirachtin (4) in THF-d_8 at 293K and 183K

	δ, 293K	δ, 183K
3'-H	7.08	7.16; 7.08
17-H	2.24	2.24; 2.10
21-H	4.50	4.85; 4.99
22-H	5.30	5.15; 4.95
23-H	6.39	5.57; 6.42
12-OCH_3	3.52	3.48; 3.42
29-OCH_3	3.75	3.77; 3.74

Structure of 22,23-Dihydro-23ß-methoxyazadirachtin (5)

The molecular formula $C_{36}H_{48}O_{17}$ of (5) was determined from the elemental analysis and the mass spectrum (FD-MS: 753, MH^+). The ^1H and ^{13}C n.m.r. data (tables 1 and 2, resp.), and the n.O.e experiments (table 3) indicated that (5) is closely related to azadirachtin (4). The positions of the hydroxy groups, particularly the 11-OH group, were determined from the deuterium isotope shifts (figure 1) and on the basis of the strong n.O.e. on 9-H and 30-H upon saturation of 11-OH (δ 5.03). 7-OH, in addition, was determined from the HCOH coupling observed in $(CD_3)_2SO$. The enhancements of 6-H, 7-H, 15-H, 11-OH, 19-H_a (negative), and 19-H_b (positive) during saturation of 30-H indicated that (5), like azadirachtin (4) possesses the oxygen bridge connecting C-11 and C-19. The signals at δ 68.38 and 69.34 were assigned to the oxiran carbons 13 and 14. Significant differences in the n.m.r. spectra of (4) and (5) were found for the following points:

(i) Instead of the olefinic protons in (4) (δ 5.05 and 6.46) an ABX system appears in the proton spectrum of (5) with doublets of doublets at δ 2.38 (22-H_α), 2.22 (22-H_β), and 5.18 (23-H), indicating a proton attached to a ketale carbon;
(ii) an additional methoxy signal is observed at δ 3.42;
(iii) the 16-H_b signal is shifted downfield by 0.6 ppm compared to (4);
(iv) in the $^1H,^{13}C$ heteroscalar correlated 2D n.m.r. spectrum of (5) the signal of C-22 [δ 107.30, d in (4)] and C-23 [δ 147.00, d in (4)] appears at δ 48.10 (t, as drawn from the DEPT spectra, C-22) and δ 105.75 (d, C-23).

The chemical shift of δ 105.75 indicated that C-23 is a ketale carbon; therefore the additional methoxy group must be connected to C-23. The configuration of 23-OCH_3 was determined by n.O.e. experiments in the FT difference spectrum. Saturation of 21-H which is in the α-configuration produces n.O.e's on 7-H, 7-OH, 20-OH, and 23-H. Enhancement of 23-H is only possible if 21-H and 23-H are located on the same side of the tetrahydrofurane ring. Thus the configuration of 23-OCH_3 must be ß. The assignment of the diastereotopic protons 22-H_α and 22-H_β was achieved by analysis of the ABX system 22-H_α, 22-H_β, and 23-H which had been determined by homodecoupling experiments. 22-H_β was assigned to the signal at δ 2.22 because of the smaller ^3J coupling to 23-H (3.2 Hz) which is consistent with an anti-clinal orientation of the two protons. Consequently 22-H_α must

be in a syn-clinal orientation relative to 23-H, according to the larger vicinal coupling constant (^3J = 6.4 Hz). Lacking of the 22,23 double bond in (5) gives rise to a strong downfield shift of the 16-H_b signal (δ 1.92) compared to (4) (δ 1.31) whereas the 16-H_a signal is almost unaffected. However, the 16-H_a signal is now found at higher field (δ 1.64) than 16-H_b (δ 1.92) in (5) where only the anisotropy of the oxiran oxygen is operative. Therefore 16-H_a and the epoxide ring must be located on the same side of the five membered ring in (4) and (5). Thus structure (5) had to be assigned to 22,23-dihydro-23ß-methoxyazadirachtin.

In order to prove whether (5) could have been formed from azadirachtin (4) during work up by addition of methanol to the 22,23 double bond (4) was treated with methanol under the conditions of the isolation and separation procedures. No dihydromethoxy derivative (5) could be detected in the reaction mixture.

Structure of 3-Deacetyl-3-cinnamoylazadirachtin (6)

The structure of 3-deacetyl-3-cinnamoylazadirachtin $C_{42}H_{48}O_{16}$ (808) was determined as (6) by FD-MS (m/z 790; M^+-H_2O) and by comparison of the n.m.r. spectra (tables 1 and 2) and the n.O.e's (table 6) with that of azadirachtin (4). The proton signals are very similar in (4) and (6) except for the ester substituent at C-3. The acetyl group of (4) is missing in (6). Instead the signals of two protons attached to a double bond in *E*-configuration (δ 6.26 and 7.65, J = 16 Hz), and a multiplet (δ 7.36-7.48) integrating for five phenyl protons are found. This indicates that a cinnamoyl ester group is present in the molecule. Like in the case of (4) and (5) the assignment of the tiglate group to C-1 and hence the cinnamate group to C-3 was achieved for (6) on the basis of the n.O.e. on 3'-H observed in addition to the n.O.e's on 9-H, 17-H, 7-OH, and 20-OH during saturation of 18-H (table 6). As in (4) and (5) the location of the C-11/C-19 oxygen bridge was proved by the enhancement of 6-H, 7-H, 15-H, and 19-H_b upon saturation of 30-H (table 6). Like (4) and (5) 3-deacetyl-3-cinnamoylazadirachtin (6) is a C-11 hemiacetal as indicated from the C-11 singlet at δ 104.54 in the ^{13}C n.m.r. spectrum and the n.O.e's 30-H/11-OH, and 9-H/11-OH.

Table 6. Nuclear Overhauser effects in the ^1H n.m.r. spectrum (250 MHz, $CDCl_3$) of 3-deacetyl-3-cinnamoylazadirachtin (6).

Irradiated	Observed
9-H	18-H
18-H	9-H, 17-H, 3'-H, 7-OH, 20-OH
21-H	7-H, 22-H, 23-H, 7-OH, 20-OH
30-H	6-H, 7-H, 15-H, 19-H_b, 11-OH

Structure of 3-Tigloylazadirachtol (7)

The molecular formula of 3-tigloylazadirachtol was determined as $C_{33}H_{42}O_{14}$ by elemental analysis, FD-MS (m/z = 662, M^+; 645, MH^+-H_2O) and by a laser microprobe mass analyser (LAMMA) spectrum (m/z 662, M^+; 645, MH^+-H_2O; 548, M^+-tigloyl-CH_3O). Structure (7) was assigned to the compound on the basis of the ^1H and ^{13}C n.m.r. data (tables 1 and 2) which are very similar to those of (4), except the following points:

(i) The signal of the acetyl group is missing;

(ii) two secondary and only one tertiary OH group are present as proved by D_2O exchange and HCOH coupling in the ^1H n.m.r. in $(CD_3)_2SO$;

(iii) no hemiacetal carbon [C-11, δ 104.10 in (4), δ 104.23 in (5), δ 104.54 in (6)] was observed in the ^{13}C n.m.r. spectrum. Instead a tertiary carbon signal appeared at δ 79.48 which was identified as C-11 by a ^1H,^{13}C correlated 2D spectrum. The corresponding proton signal was found at δ 4.47.

One of the secondary OH groups showed a broad singlet at δ 3.29 which appeared as a doublet in $(CD_3)_2SO$, and which was converted to a sharp singlet upon irradiation of 7-H. Thus this OH group

was assigned to C-7. The tertiary OH was assigned to C-20 on the basis of the n.O.e. 21-H/20-OH. The n.O.e. 3'-H/18-H which is typical for (4), (5) and (6), is missing in (7) thus indicating that the tiglate group is not attached to C-1. Therefore the remaining secondary OH groups was proposed to be at the 1-position and the tiglate group at C-3. This was proved by homodecoupling and n.O.e. measurements (table 7). 1-H was identified by a n.O.e. experiment in C_6D_6 as the solvent which allowed a better separation of the 1-H and 19-H_a signals, and using a frequency cycling method similar to the method proposed by Neuhaus[20] and Kover.[21] Saturation of 19-H_a (δ 3.44, ^2J = 9.4 Hz, in C_6D_6) produced a 20% enhancement of 19-H_b and a 5% n.O.e. on 1-H (δ 3.68, $^3J_{1\text{-H},2\text{-H}\alpha}$ = 2.3, $^3J_{1\text{-H},2\text{-H}\beta}$ = 2.7, $^3J_{1\text{-H},1\text{-OH}}$ = 5.8 Hz). The 1-H signal (δ 3.52 in $CDCl_3$) is shifted by ca 1 ppm to higher field compared to (4) (δ 4.75), (5) (δ 4.73), and (6) (δ 4.79). The OH-signal at δ 3.41 is enhanced by 11% upon saturation of 9-H, and is coupled to 1-H by HCOH coupling of 5.8 Hz. Hence the OH group is attached to C-1, not to C-3 or C-7. The n.O.e. between 9-H and the OH group is possible only if the 1-OH group is α-oriented. This α-configuration of 1-OH is further confirmed by the long range coupling to 2-H$_\beta$ ($^4J_{1\text{-OH},\ 2\text{-H}\beta}$ = 1.0 Hz). On the basis of these results the tiglate group was assigned to be attached to C-3. The determination of the oxygen bridge between C-11 and C-19, and the oxiran ring involving carbon atoms 13 (δ 66.59) and 14 (δ 69.43) was achieved *via* n.O.e. experiments (table 7), and by comparison of the ^1H and ^{13}C n.m.r. data of (4), (5), (6), and (7). Saturation of 30-H (δ 1.45) gave n.O.e's on 19-H_a (negative) and 19-H_b (positive), 6-H, 7-H, 15-H, and on the 11-H signal at δ 4.47. This signal is coupled to 9-H (^3J = 1.3 Hz) and enhanced by 3% upon saturation of 9-H. The small coupling constant is consistent with the configuration of 11-H as drawn in formula (7). N.O.e's were observed also for 9-H/18-H and, unlike (4), (5), and (6), for 18-H/30-H indicating that rotation around the C-8/C-14 bond is less hindered by hydrogen bonding in (7).

Table 7. Nuclear Overhauser effects in the ^1H n.m.r. spectrum (250 MHz, $CDCl_3$) of 3-tigloylazadirachtol (7).

Irradiated	observed
9-H	5-H, 11-H, 18-H, 1-OH
11-H	9-H, 30-H
18-H	9-H, 17-H, 30-H
21-H	30-H, 7-H, 23-H, 20-OH
30-H	6-H, 7-H, 11-H, 15-H, 19-H_a(-), 19-H_b(+)

The n.m.r. spectra of (7) were very similar to that of 3-deacetylazadirachtinol reported by Kubo[22] which prompted us to publish a reassignment of that structure as (7) according to our n.m.r. interpretation.[8] Most recently, however, a compound, 3-deacetyl-11-desoxyazadirachtin, was isolated by Ley *et al.*[19b] which is obviously identical with the one obtained by Kubo. A comparison of all available data led to the conclusion that Kubo's 3-deacetylazadirachtinol, now reassigned as 3-deacetyl-11-desoxyazadirachtin[19b], and 3-tigloylazadirachtol (7)[8] are in fact different compounds which differ only in the position of the tigloyl group.

Structure of 1-Tigloyl-3-acetyl-11-methoxy-azadirachtinin (8)

The molecular formula $C_{36}H_{46}O_{16}$ of (8) was derived from the M^+ peak (734) and the high resolution of the M^+-CH_3OH peak in the EI mass spectrum, m/z 702.2527, (calc. m/z 702.2524). The ^1H n.m.r. spectrum (table 1) showed signals of an acetoxy group, a tigloyloxy group, two methoxy substituents, and two OH groups determined by D_2O exchange. Two signals at δ 4.88 and δ 6.39 were assigned to be enol ether protons (22-H and 23-H, resp.). On the basis of the chemical shift and multiplicity of the ^{13}C n.m.r. signals (table 2) two ester carbonyl groups, ten carbon atoms attached to oxygen by single bonds and two acetal carbons were identified in addition to the olefinic carbon atoms 22 and 23. From the chemical shifts, coupling constants, homodecoupling experiments and comparison with the azadirachtin spectrum it became clear that, like in azadirachtin (4), the tiglate group is connected to C-1 and the acetate group to C-3: Irradiation at δ 4.81 (1-H) or δ 5.48 (3-H) converted the signals at δ 2.28 and δ 2.13 (2-H$_\alpha$, 2-H$_\beta$) into doublets. Saturation of the 18-H signal (δ 1.50, *vide infra*) resulted in a n.O.e. on 3-H'(table 8), thus

Table 8. Nuclear Overhauser effects in the ^1H n.m.r. spectrum (250 MHz, $CDCl_3$) of 1-tigloyl-3-acetyl-11-methoxyazadirachtinin (8).

Irradiated	Observed
7-H	21-H, 30-H, 20-OH
9-H	5-H, 18-H, 11-OCH_3
18-H	5-H, 9-H, 16-H_a, 17-H, 14-OH, 3'-H
15-H	30-H, 14-OH
21-H	7-H, 23-H, 20-OH
30-H	6-H, 7-H, 15-H, 19-H_b, 14-OH, 11-OCH_3
14-OH	7-H, 9-H, 15-H, 20-OH
20-OH	7-H, 21-H
11-OCH_3	5-H, 18-H, 30-H, 14-OH 12-OCH_3
12-OCH_3	1-H, 19-H_b, 11-OCH_3, 4'-H/5'-H

indicating that the tiglate group is connected to C-1 in α-configuration. 5-H, 6-H, and 7-H form a similar spin system as in azadirachtin (4) (table 1), the signals beeing slightly shifted to higher field, and showing axial, axial coupling for 5-H, 6-H (12.7 Hz), and axial, equatorial coupling for 6-H and 7-H (3.2 Hz). 9-H and 5-H were correlated by a 7% n.O.e. on saturation of the 9-H signal at δ 3.56. Long range coupling was observed for C-4/2-H_α, C-4/5-H, C-5/3-H, C-5/7-H, C-6/5-H, C-8/9-H, C-10/2-H_α, C-10/5-H, and C-10/9-H in the $^1H,^{13}C$ heteroscalar correlated 2D n.m.r. spectrum (table 9). The signals at δ 3.66 and 4.03 were assigned to 28-$H_{\alpha,\beta}$, according to chemical shifts and geminal coupling (^2J = 8.8 Hz) which are very similar to compounds (4) - (7) and other limonoids, containing an oxygen bridge between C-28 and C-6 (table 1)[23]. The C-8 methyl group (30-H, δ 1.57) was identified by the n.O.e's on 6-H and 7-H on irradiation at δ 1.57, the n.O.e. 7-H/30-H, and by long range couplings C-7/30-H, C-8/30-H, and C-30/9-H, determined by $^1H,^{13}C$ heteroscalar correlated 2D n.m.r. spectra. Irradiation at δ 1.57 (30-H) produced also a 7% enhancement of 19-H_b (δ 4.21) which shows geminal coupling (J = 9.7) to 19-H_a (δ 3.73), similar to 19-$H_{a,b}$ in azadirachtin (4) and it's analogues (5) - (7), and long range coupling C-1/19-H_b. C-19 (δ 70.44) was assigned *via* long range couplings to 5-H and 9-H. Hence, like in azadirachtin (4) the oxygen bridge connecting C-11 and C-19 is present also in (8). The chemical shift of C-11 (δ 107.25) indicated that C-11 is an acetal carbon. This assumption was confirmed by n.O.e. and long range couplings: Saturation of the CH_3O signal at δ 3.37 produced a n.O.e. on 30-H and *vice versa*, and long range coupling was found for C-11 and the CH_3O signal at δ 3.37. Hence this methoxy group is connected to C-11. The δ 3.72 signal was assigned to the CH_3O group attached to C-12 on the basis of the long range coupling C-11/12-OCH_3 observed in the $^1H,^{13}C$ heteroscalar correlated 2D spectrum. The remaining methoxyl (δ 3.77) belongs to the C-29 ester group. The spin system 15-H (δ 4.13), 16-$H_{a,b}$ (δ 2.15, 1.85), and 17-H (δ 2.12) cannot be analyzed as easily as in azadirachtin (4) because of overlapping signals if the ^1H n.m.r. spectrum is run in $CDCl_3$ as the solvent. A better resolution is achieved if C_6D_6 is used (table 1). The 15-H signal which appears as a doublet in the spectra of azadirachtin (4) and it's analogues (5) - (7) (table 1) is a multiplet in the spectrum of (8) because of additional coupling to 16-H_b and 17-H. $^3J_{15/16a}$ (2.5 Hz) is smaller. $^3J_{17/16a}$ bigger than for azadirachtin. The orientation of 15-H was determined from the n.O.e's 15-H/30-H and 30-H/15-H. The signals of C-15 (δ 81.42), C-16 (δ 29.63) and C-17 (δ 50.81) were assigned by a heteroscalar correlated spectrum and found to be shifted downfield compared to azadirachtin (4) (table 2). Highfield shift of the ^1H signal was observed for 18-H (δ 1.50) which was assigned on the basis of the n.O.e's 18-H/9-H (25%), and 18-H/3'-H (15%). The C-18 signal (δ 26.67) is shifted downfield by 8 ppm compared to azadirachtin. Such changes are typical for the loss of γ gauche interactions. That is an indication that the conformation in this part of (8) must be different compared to azadirachtin (4) and its analogues (5) - (7). This assumption is confirmed by the observation of a 8% enhancement of 16-H_a (δ 2.15) upon saturation of 18-H. The assignment of the ^{13}C n.m.r. signals at δ 95.10 and δ 93.26 to C-13 and C-14, resp., was achieved on the basis of the hetereoscalar correlated 2 D spectrum optimized for long range coupling (J = 10 Hz, table 10). ^3J coupling was observed for C-13 (δ 95.10) to 15-H, 16-H_a, and the hydroxy group at δ 4.33, in addition to the ^2J coupling to 18-H; C-14 (δ 93.26) shows ^3J coupling to 9-H, thus indicating a C-14/C-8 connection, and coupling to 16-H_a, 16-H_b, 18-H, and 30-H (table 9). The oxygen bridge

C-15/C-21 was determined by long range coupling C-15/21-H. The assignment of the dihydrofurane ring was accomplished on the basis of the chemical shifts in the ^{1}H and ^{13}C n.m.r. spectra, the n.O.e. enhancements 7-H/21-H, 21-H/7-H (table 8), and the long range coupling C-20/23-H, and C-23/22-H (table 9).

The chemical shifts of C-13 (δ 95.10), C-14 (δ 93.26), and C-20 (δ 86.44) are in the range expected for quarternary carbon atoms attached to hydroxy or alkoxy groups[15]. In order to localize the two hydroxy groups detected by D_2O exchange a proton n.m.r. spectrum was run in $(CD_3)_2SO$. However, no HCOH coupling could be detected; hence, no primary or secondary hydroxy group is present in the molecule. The positions of the tertiary hydroxy groups were proved by ^{13}C deuterium isotope shift experiments in $(CD_3)_2SO$. ß-Isotope shifts were found for C-14 (δ 93.26) and C-20 (δ 86.44) but not on C-7 (figure 1). This is an indication that the hydroxy groups are attached to C-14 and C-20. In addition a small γ-isotope shift on C-22 was observed (table 2). The assignment of the OH signals at δ 4.33 to 14-OH and δ 6.07 to 20-OH was achieved by n.O.e. experiments: Irradiation of 14-OH produced 6% enhancements on 15-H and 9-H, saturation of 20-OH produced enhancements on 7-H (7%) and 21-H (6%) and *vice versa* (table 8). Additional n.O.e's were found for 18-H/14-OH, 30-H/14-OH, 11-OCH_3/14-OH, 15-H/14-OH (table 8). Long range coupling was observed for C-13/14-OH in the $^{1}H,^{13}C$ heteroscalar correlated 2 D spectrum (table 9). The remaining oxygen was assigned to be connected to C-7 and C-13 on the basis of chemical shifts and multiplicities of C-7 (δ 82.70, d) C-13 (δ 95.10, s), and 7-H (δ 4.53, d, $^{3}J_{7\text{-}H,6\text{-}H}$ = 3.2 Hz). From these results structure formula (8) was deduced for 1-tigloyl-3-acetyl-11-methoxy-azadirachtinin.

Table 9. Long range coupling in the $^{1}H,^{13}C$ heteroscalar correlated 2D spectrum ($CDCl_3$) of 1-tigloyl-3-acetyl-11-methoxyazadirachtin (8).

δ, ^{13}C signals	δ, ^{1}H signals
70.26 (C-1)	4.21 (19-H_b)
66.97 (C-3)	4.81 (1-H)
52.87 (C-4)	2.28 (2-H_α), 3.16 (5-H)
36.34 (C-5)	5.48 (3-H), 4.53 (7-H)
71.93 (C-6)	3.16 (5-H)
82.70 (C-7)	1.57 (30-H)
51.46 (C-8)	1.57 (30-H), 3.56 (9-H)
49.78 (C-10)	2.28 (2-H_α), 3.16 (5-H), 3.56 (9-H)
107.25 (C-11)	3.37 (11-OCH_3), 3.72 (12-OCH_3)
95.10 (C-13)	1.50 (18-H), 1.85 (16-H_a), 4.13 (15-H), 4.33 (14-OH)
93.26 (C-14)	1.50 (18-H), 1.57 (30-H), 1.85 (16-H_a), 2.15 (16-H_b), 3.56 (9-H)
81.42 (C-15)	5.64 (21-H)
70.44 (C-19)	3.16 (5-H), 3.56 (9-H)
86.44 (C-20)	6.39 (23-H)
146.08 (C-23)	4.88 (22-H)
17.63 (C-30)	3.56 (9-H)

EXPERIMENTAL

Plant Material. - Neem bark, leaves, and seed kernels were collected and dried near Poona, India;[24] seed kernels were also collected in Togo.[24]

N.m.r. Methods. - N.m.r. measurements were performed with a Bruker WM 250 instrument at ambient temperature (299K). *^{1}H n.m.r.:* 250 MHz, *^{13}C n.m.r.:* 62.89 MHz; solvent $CDCl_3$, internal standard TMS. The *$^{1}H,^{13}C$ heteroscalar correlated 2D n.m.r. spectra* were obtained with the usual pulse sequence with phase cycling as written in the BRUKER microprogram library. Data processing was performed with standard BRUKER software. The spectral width were ± 750 Hz in f_1 and 11364 Hz in f_2 giving digital resolutions of 2.9 and 2.8 Hz per data point with a 256*2048 transform matrix, which was zerofilled to 512*4096 data points. Data were handled in power mode, and a Gauß window with Lb = -4 Hz and GB = 0.2 Hz in f_2, and Lb = -1 Hz and Gb = 0.2 Hz in f_1 was applied before transformation. The *H,C COLOC spectra* were obtained with the pulse sequence with phase cycling as written in the BRUKER microprogram library. The same parameters were used as in the $^{1}H,^{13}C$ heteroscalar correlated 2D n.m.r. spectra. The delays of the mixing period, however, were

optimized for a long range coupling of 10 Hz and 5 Hz. The *N.O.e. difference spectra* were measured with an irradiation time of 3.5 sec and a short delay before aquisition. Two dummy scans were taken before each cycle of eight scans. During aquisition of the control spectra the decoupler was placed as close as possible to the irradiated signal depending on the decoupler power used. A line broadening of 2 Hz was applied before transformation of the difference FID. To saturate extended multiplets uniformly and selectivly a method similiar to the frequency cycling of Neuhaus[20] and Köver[21] was used. The frequency of each multiplet line was stored in a frequency list and irradiated during the saturation period for 0.2 sec. This was repeated twenty times for each line, so that a total irradiation time of four seconds resulted for each line. The *DEPT-spectra* were measured with the pulse sequence described in the reference[11] with an additional purging pulse.

Melting points were determined on a Büchi SMR 20 instrument and are not corrected. ***Optical rotations*** were recorded on a Perkin Elmer Polarimeter 241, ***infrared spectra*** on a Zeiss IMR 25. ***Mass spectra*** were recorded on a Varian MAT 311 A or a Varian MAT 44 S instrument.

Isolation of (4), (5), and (7). - 3.6 kg ground neem seed kernels were stirred three times with 10 liter methanol for 24 hours. Distribution between petrol ether/methanol (1:1) of the extract followed by partition between water and ethyl acetate (1:1) of the methanolic phase and the solid residue gave 248 g crude material. Chromatography on LiChroprep Si 60, (40-63µ, dichloromethane/methanol 98:2 ⟶ 90:19), and subsequently on LiChroprep RP 18, (25-40µ, methanol/water 6:4, flow 10 ml/min), gave 2.8 g of a mixture of (4) and (5) (fraction 1, t_R = 26 min), and 2.2 g of a mixture consisting of (7) and two salannolactames[4a,25] (fraction 2, t_R = 35 min).

Azadirachtin (4). - Chromatography on LiChroprep Si 60 (25-40µ) of fraction 1 (dichloromethane/methanol 99:1 ⟶ 97:3, flow 10 ml/min), gave 325 mg of pure azadirachtin (t_R = 26 min), R_f = 0.53 (Si60, CH_2Cl_2/EtOH 100:5). $C_{35}H_{44}O_{16}$ (720), FD-MS 720 (100% M^+); ^1H and ^{13}C n.m.r. spectra see tables 1 and 2, resp.

$$[\alpha]^{\lambda}_{20°C} = \frac{589}{-65.4}\ \frac{578}{-69.8}\ \frac{546}{-79.6}\ \frac{436}{-135.2}\ \frac{405}{-161.1} \quad (CHCl_3,\ c = 0.2)$$

Treatment of Azadirachtin (4) with Methanol. - a) 10 mg azadirachtin were dissolved in methanol, then 3 mg silica gel Si 60 were added. After 24 hours refluxing the solution was checked by TLC and ^1H-n.m.r. spectroscopy. No dihydromethoxy derivative (5) was detected. b) 10 mg azadirachtin were dissolved in methanol, then 3 mg silica gel Si 60 were added. After 24 hours stirring at 40°C the solution was checked by TLC and ^1H n.m.r. spectroscopy. No dihydromethoxy derivative (5) was detected.

22,23-Dihydro-23ß-methoxyazadirachtin (5). - Chromatography on LiChroprep Si 60 (25 - 40µ) of fraction 1 (dichloromethane/methanol 99:1 ⟶ 97:3) followed by chromatography of the slower fractions (t_R = 32 - 35 min) on LiChroprep RP 18 (10µ) with methanol/water 1:1 (flow 5 ml/min) gave crude (5), t_R = 44 min, which was purified by chromatography on Spherisorb CN (5µ) with petrol ether/dichloromethane/methanol 100:10:10 (flow 5 ml/min.) to give 170 mg amorphous (5), t_R = 10.5 min, R_f = 0.42 (Si60, CH_2Cl_2/EtOH 100:5). Found C 56.85%, H 6.51%, $C_{36}H_{48}O_{17}$ (762.8) requires C 57.44%, H 6.42%. IR (KBr): 3440 (OH); 2850 (OCH_3); 1745, 1735, 1720, 171 (C=O); 1270, 1220, 1075, 1035, C-O). FD-MS: m/z 753 (55%, MH^+-H_2O), 720 (78%), 693 (55%), 689 (24%), 674 (100%), 661 (6%). ^1H and ^{13}C n.m.r. spectra see tables 1 and 2, resp.

$$[\alpha]^{\lambda}_{20°C} = \frac{589}{-8.1}\ \frac{578}{-8.5}\ \frac{546}{-9.2}\ \frac{436}{-14.9}\ \frac{405}{-18.0}\ \frac{365}{-23.6} \quad (CHCl_3,\ c = 0.1)$$

3-Tigloylazadirachtol (7). - Chromatography on LiChroprep Si 60 (25-40µ) of fraction 2 (2.8g) with dichloromethane/methanol 100:1 ⟶ 100:3 (flow 10 ml/min) followed by chromatography on LiChrosorb Si 60 (7µ) with petrol ether/dichloromethane/methanol 100:10:8 (flow 6 ml/min), and purification on LiChrosorb RP 18 (10µ) with methanol/water 6.5:3.5 (flow 5 ml/min) gave 20 mg (7), t_R = 31 min, R_f = 0.68 (CH_2Cl_2/EtOH 100:5), m.p. 204-206°C (needles, from water/ethanol 100:1). Found C 59.06%, H 6.20%, $C_{33}H_{42}O_{14}$ (662.7) requires C 59.81%, H 6.38%. IR (KBr): 3560, 342, (OH); 3040 (=C-H); 1735, 1725, 1720, 1710, (C=O); 1650, 1610 (enone, enol ether); 1270, 1230, 1135, 1075 (C-O). FD-MS: m/z 662 (70%, M^+); 645 (100%, MH^+-H_2O). LAMMA spectrum:[26] m/z 662, 645, 548 (M^+-tigloyl-CH_3O). ^1H and ^{13}C n.m.r. spectra see tables 1 and 2, resp.

$$[\alpha]^{\lambda}_{20°C} = \frac{589}{-69.4}\ \frac{578}{-70.8}\ \frac{546}{-77.7}\ \frac{436}{-125.0}\ \frac{405}{-151.4}\ \frac{365}{-204.2} \quad (CH_2Cl_2,\ c = 0.1)$$

Isolation of 3-Deacetyl-cinnamoylazadirachtin (6). - Soxhlet extraction (30 hours) with diethyl ether of 10.5 kg defatted (petrol ether, b.p. 30-50°C) and powdered neem leaves gave 326 g raw material. 287 g of the extract were dissolved in 1.7 l diethyl ether, stirred for 95 min. with 291 g charcoal, and kept over night. After filtration and removal of the solvent 250 g extract were obtained. Chromatography of the material on 2 kg silica gel Si 60 (65-200µ) with petrol ether/ethyl acetate 9:1 (4.2 l), 8:2 (7.8 l) 7:3 (10.2 l), 6:4 (11.4 l), 1:1 (18 l), yielded fractions consisting of 6-hydroxyazadiron[4c] and several meldenin derivatives.[4c] Further elution with ethyl acetate (18.6 l) gave a fraction (33 g) consisting of (6), 4α,6α-dihydroxy-A-homoazadiron,[27] and 1,2-dihydro-11-acetyl-4α,6α-dihydroxy-A-homoazadiron.[4c] Repeated chromatography on silica gel Si 60 (15-25µ) with dichloromethane/ethanol 97:3 (flow 19 ml/min, UV detection 254 nm, t_R = 20 min), LiChroprep RP 18 (15-25µ, Latek glass column) with methanol/water 7.4 : 2.6 (flow 10 ml/min, UV detection 254 nm, 10.5 g, t_R = 8 min), and LiChroprep RP 18 (15-25µ) with methanol/water 7:3 (flow 8 ml/min, UV detection 254 nm) gave 3-deacetyl-3-cinnamoyl azadirachtin (6), t_R = 81 min, which was purified by HPLC on silica gel Si 60 (7µ) with petrol ether/dichloromethane/ethanol 9:1:1 (flow 4.5 ml/min, UV detection 254 nm). Yield 48 mg (6), t_R = 58 min, R_f =

0.49 (CH_2Cl_2/EtOH 100:5). $C_{42}H_{48}O_{16}$ (808), FD-MS m/z 790 (M^+-H_2O), 677 ($M^+-C_8H_7CO$), 151, 131 ($C_8H_7CO^+$), 95, 83 ($C_4H_7CO^+$). IR (CCl_4): 3420 (OH), 1735, 1720 (C=O), 1635 (C=C); 1270, 1160 cm^{-1} (C-O). ^1H and ^{13}C n.m.r. spectra see tables 1 and 2, resp.

$$[\alpha]^{\lambda}_{25°C} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 405}{3.7 \quad 3.5 \quad 4.9 \quad 20.6 \quad 32.2} \quad (CHCl_3,\ c = 0.1)$$

Isolation of 1-Tigloyl-3-acetyl-11-methoxyazadirachtinin (8). - 20 kg powdered neem bark were extracted with ether in a Soxhlet apparatus. After evaporation of the solvent the extract was partitioned between equal amounts of petrol ether (30-50°C) and methanol/water (95:5). Work up of the methanolic phase gave 270 g residue which on chromatography on 4.5 kg silica gel with petrol ether/ethyl acetate (90:19 ⟶ 0:100) followed by reversed phase chromatography on RP 18 with methanol water (3:1) yielded 160 mg amorphous (8), R_f = 0.54 (Si60, CH_2Cl_2/EtOH 100:5). $C_{36}H_{46}O_{16}$ (734.3). EI-MS: Found 702.2527, M^+-CH_3OH requires 702.2524. IR (KBr) 3040 (OH), 1745, 1710, (C=O), 1650, 1630 (C=C), 1270, 1250, 1240, 1160, 1130, 1100, 1050 cm^{-1} (C-O). EI-MS: m/z 734 (M^+), 675 (M^+-CH_3COO), 702 (M^+-CH_3OH). ^1H and ^{13}C n.m.r. spectra see tables 1 and 2, resp.

$$[\alpha]^{\lambda}_{25°C} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 404 \quad 365 \quad 313}{-1.1 \quad -1.2 \quad -1.3 \quad -2.4 \quad -2.9 \quad -4.0 \quad -5.6} \quad (CHCl_3,\ c = 0.1)$$

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