3-Tigloylazadirachtol (Tigloyl = 2-Methylcrotonoyl), an Insect Growth Regulating Constituent of *Azadirachta indica*

Adolf Klenk, Michael Bokel, and Wolfgang Kraus*

Institut für Chemie der Universität Hohenheim, Lehrstuhl für Organische Chemie, 7000 Stuttgart 70, West Germany

The structure of deacetylazadirachtinol (**2**) has been reassigned as 3-tigloylazadirachtol (**3**) (tigloyl = 2-methylcrotonoyl) on the basis of a detailed ^1H and ^{13}C n.m.r. spectroscopic analysis.

In connection with our work on constituents of *Azadirachta indica* we recently were able to reassign the structure of the insect antifeedant azadirachtin[1] as (**1**).[2,3] These results prompted us to reinvestigate the n.m.r. analysis of deacetylazadirachtinol (**2**),[4] which, from inspection of the spectra, is closely related to azadirachtin. The results summarized in Tables 1, 2, and 3 lead us to propose the structure (**3**) for this compound which we suggest to be named 3-tigloylazadirachtol (tigloyl = 2-methylcrotonoyl).

Compound (**3**) was isolated by chromatography of methanolic neem seed extracts and recrystallized from ethanol–water mixtures; m.p. 204—206 °C, $[\alpha]_D^{20}$ −69° (*c* 0.1). The elemental analysis, the field desorption mass spectrum *m/z* 662 (M^+), 645 (MH^+-H_2O), and the laser microprobe mass analyser (LAMMA) spectrum *m/z* 662, 645, 548 (M^+ − tigloyl − MeO) indicated the molecular formula $C_{33}H_{42}O_{14}$ and not $C_{33}H_{44}O_{15}$ [soft ionization mass spectrum *m/z* 645 ($M-2H_2O+H)^+$] as proposed by Kubo *et al.*[4]

As described previously no signal of a hemiacetal carbon (C-11, δ 104.10 in azadirachtin) was observed in the ^{13}C n.m.r. spectrum. Instead a tertiary carbon signal appeared at δ 79.48, which could be identified as C-11 by a ^1H–^{13}C heteroscalar correlated 2D n.m.r. spectrum.[5] The corresponding proton signal was found at δ 4.47 in full agreement with the literature.[4] All other ^1H n.m.r. chemical shifts of (**3**) (Table 1) are identical with the published values.[4] Some assignments however could be corrected on the basis of simple homodecoupling experiments and nuclear Overhauser effects (n.O.e.) in the Fourier transform difference spectrum.

In contrast to the structure proposed by Kubo *et al.* we determined the tiglate group to be at the 3-position. This was done by n.O.e. experiments with saturation of 19-H_a (δ 3.44, $^2J_{19\text{-}H_a,19\text{-}H_b}$ 9.4 Hz, in C_6D_6). For this and the following experiments C_6D_6 was used as the solvent, because of the better separation of 19-H_a and 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). To irradiate 19-H_a uniformly,

Table 1. 250 MHz ^1H N.m.r. data ($CDCl_3$) for azadirachtin (**1**) and 3-tigloylazadirachtol (**3**) (*J* in Hz).

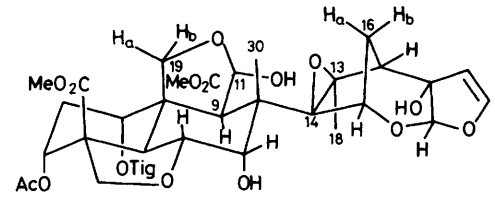
	(1)	(3)
1-H	4.75 (dd, *J* 2.9,3.1)	3.52 (ddd, *J* 2.3,2.7,5.8)[a]
2-Hα	2.34 (ddd, *J* 16.7,2.9,2.7)	3.32 (ddd, *J* 16.3,2.3,2.8)
2-Hβ	2.13 (ddd, *J* 16.7,3.1,2.9)	2.06 (dddd, *J* 16.3,2.7,2.8,1.0)
3-H	5.50 (dd, *J* 2.7, 2.9)	5.53 (dd, *J* 2.8,2.8)
5-H	3.35 (d, *J* 12.5)	3.33 (d, *J* 12.7)
6-H	4.60 (dd, *J* 12.5,2.7)	4.55 (dd, *J* 12.7,2.6)
7-H	4.75 (d, *J* 2.7)	4.72 (d, *J* 2.6)
9-H	3.34 (s)	3.19 (d, *J* 1.3)
11-H	—	4.47 (d, *J* 1.3)
15-H	4.67 (d, *J* 3.4)	4.58 (d, *J* 3.9)
16-H_a	1.73 (ddd, *J* 13.0, 3.4,5.1)	1.65 (ddd, *J* 12.9,3.9,5.3)
16-H_b	1.31 (d, *J* 13.0)	1.33 (d, *J* 12.9)
17-H	2.38 (d, *J* 5.1)	2.36 (d, *J* 5.3)
18-H	2.01 (s)	2.04 (s)
19-H_a	3.63 (d, *J* 9.6)	3.49 (d, *J* 9.4)[b]
19-H_b	4.15 (d, *J* 9.6)	3.95 (d, *J* 9.4)
21-H	5.65 (s)	5.66 (s)
22-H	5.05 (d, *J* 2.9)	5.03 (d, *J* 2.9)
23-H	6.46 (d, *J* 2.9)	6.43 (d, *J* 2.9)
28-Hα,β	4.08 (d, *J* 9.0)	3.83 (d, *J* 9.0)
	3.76 (d, *J* 9.0)	4.04 (d, *J* 9.0)
30-H	1.74 (s)	1.45 (s)
1-OH	—	3.41 (dd, *J* 5.8,1.0)
7-OH	3.02 (br s)	3.29 (br s)
11-OH	5.05 (s)	—
20-OH	3.11 (br s)	2.78 (br s)
$MeCO_2$	1.95 (s)	—
CO_2Me	3.76 (s)	3.76 (s)
	3.68 (s)	3.76 (s)
Tigloyl		
3′-H	6.93 (qq, *J* 7.0,1.5)	6.95 (qq, *J* 7.0,1.3)
4′-H	1.78 (dq, *J* 7.0,1.1)	1.79 (dq, *J* 7.0,1.3)
5′-H	1.85 (dq, *J* 1.5,1.1)	1.84 (dq, *J* 1.3,1.3)

[a] In C_6D_6 this signal is at δ 3.68. [b] In C_6D_6 this signal is at δ 3.44.

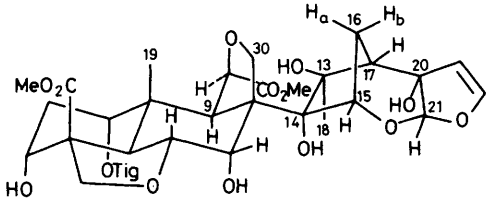
Table 2. 62.89 MHz ^{13}C N.m.r. data ($CDCl_3$) of azadirachtin (**1**) and 3-tigloylazadirachtol (**3**).

	(1)	(3)
C-1	70.51 d	69.37 d
C-2	29.37 t	32.09 t
C-3	66.99 d	67.69 d
C-4	52.52 s	53.34 s
C-5	37.06 d	35.18 d
C-6	73.79 d	74.32 d
C-7	74.37 d	73.68 d
C-8	45.59 s	44.04 s
C-9	44.69 d	43.80 d
C-10	50.19 s	51.24 s
C-11	104.10 s	79.48 d
C-12	171.70 s	173.52 s[a]
C-13	68.53 s	66.59 s
C-14	69.69 s	69.43 s
C-15	76.43 d	76.16 d
C-16	25.06 t	25.08 t
C-17	48.67 d	48.99 d
C-18	18.49 q	18.56 q
C-19	69.07 t	71.43 t
C-20	83.55 s	83.71 s
C-21	108.70 d	109.16 d
C-22	107.30 d	107.53 d
C-23	147.00 d	146.79 d
C-28	72.99 t	73.30 t
C-29	173.20 s	174.12 s[a]
C-30	21.33 q	21.35 q
CO_2Me	53.52 q	53.01 q
	52.72 q	52.63 q
$MeCO_2$	169.50 s	
$MeCO_2$	20.88 q	
Tigloyl		
C-1′	166.10 s	167.08 s
C-2′	128.60 s	128.49 s
C-3′	137.50 d	138.86 d
C-4′	14.29 q	14.69 q
C-5′	11.94 q	12.09 q

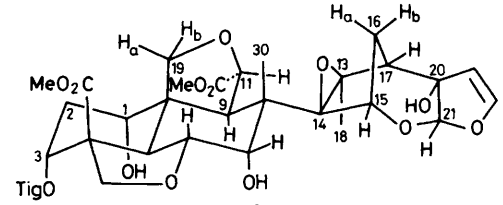
[a] Assignments could be reversed.



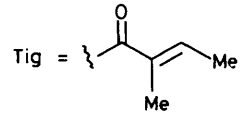
(1)



(2)



(3)



a frequency cycling method similar to the methods proposed by Neuhaus[6] and Köver[7] was used.

In this experiment a 20% n.O.e. was observed for 19-H_b and a 5% n.O.e. for 1-H. The signal at δ 3.41 is enhanced by 11% upon saturation of 9-H, and is coupled to 1-H by a HCOH coupling of 5.8 Hz. Thus this OH group is attached to C-1, not to C-3 or C-7. Saturation of 9-H also gave n.O.e.s on 11-H (3%) and 18-H (11%). The n.O.e. between 9-H and the hydroxy group is possible only for an α-oriented OH attached to C-1. Therefore the tiglate group must be attached to C-3 and the assignment of 1-H and 3-H is reversed. The α-orientation of 1-OH is further confirmed by the ^4J coupling of 1-OH to 2-Hβ ($^4J_{1\text{-OH},2\text{-H}\beta}$ 1.0 Hz).

During saturation of the methyl group at δ 1.45 n.O.e.s of 6-H, 7-H, 11-H, and 15-H could be observed (Table 3). Furthermore a positive effect of this methyl group on one of the methylene protons at δ 3.95 and a negative one on the other at δ 3.49 lead us to assign the methyl group to 30-H and the methylene protons to 19-H_b and 19-H_a, respectively. Protons 30-H, 19-H_a, and 19-H_b are a linear three proton arrangement.[8] The absence of any n.O.e. of 1-H, 2-H, and 3-H during saturation of the methyl group signal at δ 1.45 is only consistent with the structure (**3**) containing the ether bridge between C-11 and C-19. The same experimental results were obtained for azadirachtin except for the n.O.e. between 30-H and 11-H, which allowed the configuration of C-11 to be determined as shown in (**3**). This is also consistent with the small vicinal coupling constant $^3J_{11\text{-H},9\text{-H}}$ 1.3 Hz in (**3**).

As in azadirachtin, a strong n.O.e. between 7-H and 21-H in (**3**) was observed. This and the nearly identical chemical shifts and coupling constants of the four spin system 15-H, 16-H_a, 16-H_b, 17-H lead us to assume identical structures of (**3**) and (**1**) for ring D and the dihydrofuran ring. This is strongly supported by the ^{13}C n.m.r. chemical shifts of C-13 (δ 66.59) and C-14 (δ 69.43) (Table 2). Both values are similar to those measured for the same carbon atoms in azadirachtin and are typical of quaternary oxirane carbon atoms.[2,9] The strong n.O.e. between 7-H and 21-H is only consistent with a β-oriented epoxide. Any change in the configuration of C-13 and C-14 brings about a longer distance between 7-H and 21-H, which is not consistent with this strong n.O.e.

Table 3. N.O.e. in the ^1H n.m.r. spectrum (250 MHz, $CDCl_3$) of 3-tigloylazadirachtol (**3**).

Irradiated signals/δ	Observed signals/δ
1.45 (30-H)	3.49 (negative, 19-H_a), 3.95 (positive, 19-H_b), 4.47 (11-H), 4.55 (6-H), 4.58 (15-H), 4.72 (7-H)
2.04 (18-H)	1.45 (30-H), 2.36 (17-H), 3.19 (9-H)
3.19 (9-H)	2.04 (18-H), 3.33 (5-H), 4.47 (11-H), 3.41 (1-OH)
4.47 (11-H)	1.45 (30-H), 3.19 (9-H)
5.66 (21-H)	1.45 (30-H), 4.72 (7-H), 6.43 (23-H), 2.78 (20-OH)

Reassignment of the quaternary carbon atoms C-4, -8, and -10 was possible using the values of the corresponding carbon atoms in azadirachtin, where they were unequivocally assigned by a H,C-COLOC[10] experiment. All multiplicities in the ^{13}C n.m.r. spectrum were determined by the DEPT technique.[11] On the basis of these n.m.r. results and the mass spectrum we suggest structure (**3**) for 3-tigloylazadirachtol.

We thank Professor S. V. Ley, Imperial College, for communicating his results to us prior to publication,[3] and Dr. P.-H. Wieser, University of Hohenheim, Institut für Physik, for recording the LAMMA spectrum.

Received, 18th November 1985; Com. 1622

References

1 J. H. Butterworth, E. D. Morgan, and G. R. Percy, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2445; P. R. Zanno, I. Miura, K. Nakanishi, and D. L. Elder, *J. Am. Chem. Soc.*, 1975, **97**, 1975.

2 W. Kraus, M. Bokel, A. Klenk, and H. Pöhnl, *Tetrahedron Lett.*, 1985, **26**, 6435; H. Pöhnl, dissertation, Universität Hohenheim, 1985.

3 The structure (**1**) has been confirmed recently by an *X*-ray analysis of 1-detigloyl ?3-dihydroazadirachtin: H. B. Broughton, S. V. Ley, A. M. Z awin, and D. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1986, 46.

4 I. Kubo, T. Matsumoto, and J. N. Shoolery, *Tetrahedron Lett.*, 1984, **25**, 4729.

5 R. Freeman and G. A. Morris, *J. Chem. Soc., Chem. Commun.*, 1978, 684; A. Bax and G. A. Morris, *J. Magn. Reson.*, 1981, **42**, 501.

6 D. Neuhaus, *J. Magn. Reson.*, 1983, **53**, 109.

7 K. E. Köver, *J. Magn. Reson.*, 1984, **59**, 485.

8 J. D. Mersh and J. K. M. Sanders, *Org. Magn. Reson.*, 1982, **18**, 122; J. H. Noggle and R. E. Shirmer in 'The Nuclear Overhauser Effect, Chemical Application,' Academic Press, New York, 1971, pp. 57—69.

9 F. W. Wehrli and T. Nishida, *Fortschr. Chem. Org. Naturst.*, 1979, **36**, 84, and references cited therein; W. Kraus, K. Kypke, M. Bokel, W. Grimminger, G. Sawitzki, and G. Schwinger, *Liebigs Ann. Chem.*, 1982, 87.

10 H. Kessler, L. Griesinger, J. Zarbock, and H. R. Loosli, *J. Magn. Reson.*, 1984, **57**, 331.

11 M. R. Bendall, D. M. Doddrell, and D. T. Pegg, *J. Am. Chem. Soc.*, 1981, **103**, 4603; D. M. Doddrell, R. M. Lynden-Bell, and J. M. Bulsing, *J. Magn. Reson.*, 1983, **53**, 355.