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THE STRUCTURE OF AZADIRACHTIN AND 22,23-DIHYDRO-23β-METHOXYAZADIRACHTIN

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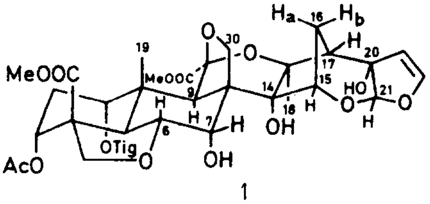
Summary: On the basis of NMR data including NOE experiments, ^{13}C deuterium isotope shifts, and ^{13}C-^{1}H long range coupling we suggest structure 2 and 3 for azadirachtin, and 22,23-dihydro-23β-methoxyazadirachtin, resp.

Azadirachtin3) is one of the most interesting constituents of Azadirachta indica because of its influence on insect feeding behaviour and insect development. Structure 1 was suggested by Nakanishi4) on the basis of NMR data. In connection with our research on constituents of Neem1) and related species2) we carried out extensive NMR measurements on azadirachtin analogues5) which prompted us to reinvestigate the NMR analysis of azadirachtin itself. The results summarized in tables 1 and 2 lead us to propose structure 2 for azadirachtin, and structure 3 for 22,23-dihydro-23β-methoxyazadirachtin6).



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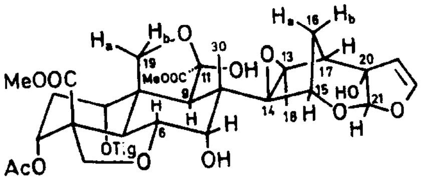

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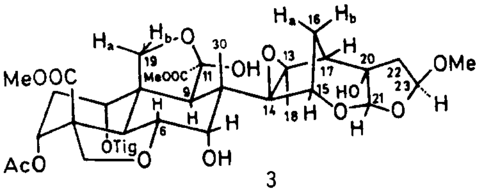

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Most of the ^{1}H and ^{13}C NMR signals of 2 (table 1) are in accord with the published values4) except the 7-H and 15-H signal which have to be reassigned on the basis of homodecoupling experiments: Irradiation at δ 4.60 (6-H) converted the doublets at δ 3.35 (5-H) and δ 4.75 into singlets. The doublet at δ 4.67 shows coupling with 16-H_a (J=3.4). Hence δ 4.75 corresponds to 7-H, δ 4.67 to 15-H. In this connection we carried out a series of NOE experiments (table 2). Saturation of the 15-H signal (δ 4.67) resulted in a strong NOE on δ 1.74 (integrating for 3 protons), 16-$H_{a,b}$, 21-H, and 11-OH. Saturation of the signal at δ 1.74 gave NOE's on 6-H, 7-H, 15-H, and 11-OH in addition to a positive effect on δ 4.15, and a negative effect on δ 3.637). There is no effect on the signals of 1-H and 2-H_β which one should expect if the signal at δ 1.74 would correspond to 19-H4). Thus the oxygen bridge is located between C-11 and C-19.

The results of the ^1H NMR analyses led to a partial reassignment of the ^{13}C NMR signals carried out by ^{13}C-^1H 2D heteroscalar correlated spectra[8a]: The C-7 signal is now found at δ 74.37 ppm instead of δ 76.43 ppm which corresponds to C-15. The signal at δ 69.07 has to be assigned to C-19 whereas the C-30 signal appears at δ 21.33. Long range coupling (COLOC experiment [8b]) was observed for C-7/30-H, C-11/19-H_a, C-11/11-OH, C-13/18-H, C-14/18-H, C-14/30-H, and C-15/21-H.

The strong NOE on 21-H observed during saturation of 7-H (table 2) is not consistent with structure formula 1, and lead us to search for alternative structures. The key problems to be solved in this connection are the positions of the hydroxy groups. 7-OH has been determined by homodecoupling experiments in $(CD_3)_2SO$ as the solvent[4]. The remaining hydroxy groups were assigned on the basis of ^{13}C deuterium isotope shift experiments which are frequently used in carbohydrate ^{13}C NMR spectroscopy[9]. Since carbon chemical shifts depend strongly on the experimental conditions the hydroxy groups were deuterated only partially, by adding small amounts of D_2O. Under these conditions the signals of both isotopomers were observed (figure 1). This method allowed to unequivocally assign 7-OH and 20-OH. In additions there is observed a distinct isotope effect also on C-11 (δ 104.07/103.97) which in earlier work[4] has been assumed to be an acetal carbon. There is no isotope effect on C-14 (δ 70.39), thus the third hydroxy group is attached to C-11. The chemical shift of C-11 corresponds very well to those found for similar hemiacetal carbons present in certain quassinoids[10]. Consequently C-11 is part of a hemiacetal structure, and there is no oxygen bridge connecting C-11 and C-13. This assignment is supported by the strong NOE on 9-H and 30-H during saturation of 11-OH in 2 and 3 (table 2). The chemical shifts of C-13 (δ 68.53) and C-14 (δ 69.69) are typical for quarternary oxiranecarbon atoms[11]. On the basis of these results we suggest structure 2 for azadirachtin bearing the epoxy oxygen in β-configuration according to the chemical shift of the 16-H_a signal in 2 and 3 (vide infra), and the strong NOE 7-H/21-H, and 7-OH/21-H.

22,23-Dihydro-23β-methoxyazadirachtin (3), $C_{36}H_{48}O_{17}$ (FD-MS:m/z 753, MH^+), $[\alpha]_D^{20} = -8.1$ (c=0.1, $CHCl_3$), was isolated by HPLC from the azadirachtin containing fractions[1,5]. The ^1H and ^{13}C NMR and NOE data in tables 1 and 2 indicate the close relationship between 2 and 3. Most of the proton signals of 2 and 3 are nearly indentical exept for the following points: (i) The signals of olefinic protons are not present anymore. Instead three new signals appear at δ 2.38 (dd), δ 2.22 (dd), and δ 5.18 (dd), which form an ABX system (22-H_α, 22-H_β, 23-H_α), as could be determined by homodecoupling experiments. (ii) A new methoxy signal appears at δ 3.42 (s). (iii) The 16-H_b signal is shifted down field by 0.6 ppm in comparison to 2.

The ^{13}C NMR resonances were assigned by a 2D heteroscalar correlated spectrum[8]: Only two signals differ significantly from the δ values in the azadirachtin spectrum: (i) δ 48.10 (triplet, as drawn from the DEPT spectra[12]) has to be assigned to C-22, δ 105.75 to C-23. The configuration of the methoxy group follows from the NOE on 23-H (table 2) caused by saturation of 21-H which is α-configurated. Thus the orientation of 23-OCH_3 must be β.

Lacking of the 22,23 double bond in 3 gives rise to a strong down field shift of the 16-H_b signal (δ 1.92) compared to 2 (δ 1.31) whereas the 16-H_a signal is almost unaffected. However, the 16-H_a resonance signal is now found at higher field than 16-H_b in 3, where only the anisotropy of the oxiran oxygen is operative. Hence, 16-H_a and the epoxide ring must be located on the same side of the five membered ring in both compounds.

The location of the OH groups was determined from the deuterium isotope shifts in the ^{13}C NMR spectra (fig. 1) as described for azadirachtin (2) (vide supra), and on the basis of the NOE 11-OH/9-H and 11-OH/30-H (table 2)[13].

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Table 1. 250 MHz ^{1}H NMR data ($CDCl_3$, TMS=0) and 62.89 MHz ^{13}C NMR data ($CDCl_3$ and $(CD_3)_2SO/D_2O$, TMS=0) of 2 and 3.

^{1}H	2	3
1-H	4.75(dd,2.9;3.1)	4.73(dd,2.6;2.6)
2-H(α)	2.34(ddd,16.7;2.9;2.7)	2.31(ddd,16.5;2.6;2.7)
2-H(β)	2.13(ddd,16.7;3.1;2.9)	2.22(ddd,16.5;2.6;2.9)
3-H	5.50(dd,2.7;2.9)	5.49(dd,2.7;2.9)
5-H	3.35(d,12.5)	3.29(d,12.4)
6-H	4.60(dd,12.5;2.7)	4.57(dd,12.4;2.5)
7-H	4.75(d,2.7)	4.65(d,2.5)
9-H	3.34(s)	3.30(s)
15-H	4.67(d,3.4)	4.67(d,3.4)
16-H(a)	1.73(ddd,13.0;3.4;5.1)	1.64(ddd,13.1;3.4;5.2)
16-H(b)	1.31(d,13.0)	1.92(d,13.1)
17-H	2.38(d,5.1)	2.47(d,5.2)
18-H	2.01(s)	2.00(s)
19-H(a)	3.63(d,9.6)	3.63(d,9.7)
19-H(b)	4.15(d,9.6)	4.15(d,9.7)
21-H	5.65(s)	5.51(s)
22-H	5.05(d,2.9)	(α) 2.38(dd,14.7;6.7) (β) 2.22(dd,14.7;3.2)
23-H	6.46(d,2.9)	5.18(dd,6.7;3.2)
28-H(α,β)	4.08(d,9.0) 3.76(d,9.0)	4.06(d,8.9) 3.74(d,8.9)
30-H	1.74(s)	1.76(s)
7-OH	2.89(br.s)	2.79(br.s)
11-OH	5.05(s)	5.02(s)
20-OH	2.92(br.s)	2.97(br.s)
3'-H	6.93(qq,7.0;1.5)	6.89(qq,7.0;1.5)
4'-H	1.78(dq,7.0;1.1)	1.77(dq,7.0;1.1)
5'-H	1.85(dq,1.5;1.1)	1.85(dq,1.5;1.1)
CH_3COO	1.95(s)	1.95(s)
$COOCH_3$	3.76(s) 3.68(s)	3.79(s) 3.67(s)
23-OCH_3		3.42(s)

^{13}C	2		3	
	$CDCl_3$	$(CD_3)_2SO/D_2O$	$CDCl_3$	$(CD_3)_2SO/D_2O$
C-1	70.51(d)	70.88	70.39(d)	70.88
C-2	29.37(t)	28.68	29.74(t)	28.83
C-3	66.99(d)	66.91	66.94(d)	66.95
C-4	52.52(s)	51.96	52.43(s)	52.52
C-5	37.06(d)	36.10	37.19(d)	36.64
C-6	73.79(d)	73.90	73.82(d)	74.16
C-7	74.37(d)	73.82 73.71	74.19(d)	73.71 73.60
C-8	45.41(s)	45.89	45.07(s)	46.02
C-9	44.69(d)	44.52	44.69(d)	44.59
C-10	50.19(s)	50.24	50.05(s)	50.16
C-11	104.10(s)	104.07 103.97	104.23(s)	104.12 104.22
C-12	171.70(s)	171.36	171.68(s)	171.49
C-13	68.53(s)	67.93	68.38(s)	67.91
C-14	69.69(s)	70.39	69.34(s)	69.42
C-15	76.43(d)	75.60	77.05(d)	77.11
C-16	25.06(t)	25.72	24.47(t)	25.79
C-17	48.67(d)	47.47	49.95(d)	52.00
C-18	18.49(q)	18.16	18.39(q)	18.07
C-19	69.07(t)	68.54	69.09(t)	68.47
C-20	83.55(s)	81.78 81.69	80.96(s)	80.24 80.33
C-21	108.70(d)	108.73	106.87(d)	107.14
C-22	107.30(d)	107.13	48.10(t)	48.68
C-23	147.00(d)	145.96	105.75(d)	105.73
C-28	72.99(t)	71.74	73.01(t)	71.98
C-29	173.20(s)	173.56	173.25(s)	173.69
C-30	21.33(q)	21.46	21.26(q)	21.92
C-1'	166.10(s)	166.22	166.15(s)	166.25
C-2'	128.60(s)	128.00	128.67(s)	127.90
C-3'	137.50(d)	137.85	137.41(d)	138.14
C-4'	14.29(q)	13.96	14.29(q)	14.07
C-5'	11.94(q)	11.54	11.94(q)	11.58
$CH_3\underline{C}OO$	169.50(s)	169.33	169.57(s)	169.85
$\underline{C}H_3COO$	20.88(q)	20.36	20.86(q)	20.50
$COO\underline{C}H_3$	53.52(q) 52.72(q)	52.38 52.05	52.22(q) 52.77(q)	54.34 52.48
23-OCH_3			55.79(q)	54.34

Table 2. Nuclear Overhauser effects in the ^{1}H NMR spectra (250 MHz, $CDCl_3$) of azadirachtin (2) and 22,23-dihydro-23β-methoxy-azadirachtin (3)

Irradiated	2 observed	3 observed
15-H	16-H(a),16-H(b),21-H,30-H,11-OH	16-H(a),16-H(b),21-H,30-H,11-OH,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-H	21-H,30-H,7-OH,20-OH	16-H(a),16-H(b),21-H,30-H,11-OH,7-OH,20-OH
21-H	7-H,7-OH,20-OH	7-H,7-OH,20-OH,23-H
18-H	9-H,17-H,3'-H,7-OH,20-OH	9-H,17-H,3'-H,7-OH,20-OH
11-OH	23-H**),15-H,9-H,30-H	9-H,30-H
7-OH	21-H,7-H,5-H	21-H,7-H,5-H
20-OH	21-H	21-H,18-H

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

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

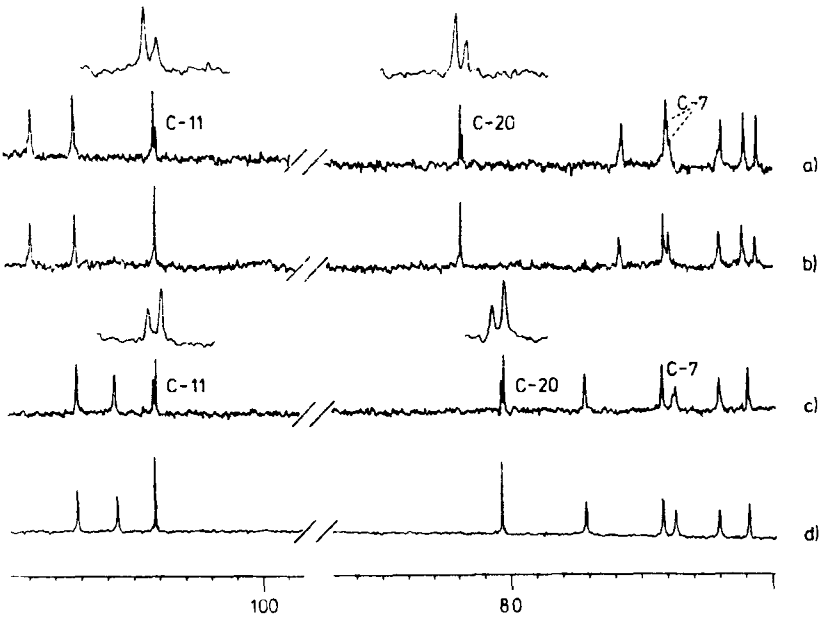

Figure 1. ^{13}C deuterium isotope shifts of C-7, C-11, and C-20: a) Azadirachtin (2) in $(CD_3)_2SO/D_2O$, b) 2 in $(CD_3)_2SO$, c) 22,23-Dihydro-23β-methoxyazadirachtin (3) in $(CD_3)_2SO/D_2O$, d) 3 in $(CD_3)_2SO$

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13) Meanwhile structure 2 has been supported by a X-ray study on the 1-detigloyl-22,23-dihydro derivative. We thank Prof. S.V. Ley, Imperial College, London, for kindly communicating his results to us.

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