Structure of Azadirachtin B

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Dedicated to Professor Erich Hecker on the occasion of his 60th birthday

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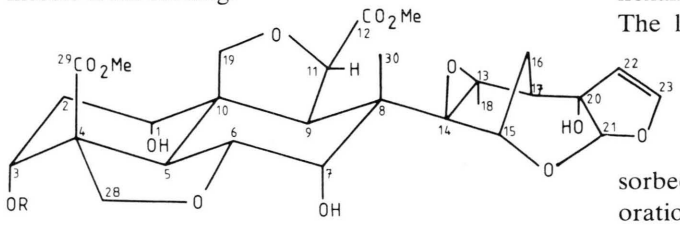
The structures of azadirachtin B and of detigloylazadirachtin have been determined on the basis of detailed ^{1}H and ^{13}C NMR spectroscopic analysis. Comparison of the data from both compounds unequivocally proves esterification by tiglic acid at position 3. Our data thus confirm the structure as proposed by Klenk *et al.*, J. Chem. Soc., Chem. Commun. **1986,** 523–524.

In the course of our studies on such allelochemicals which interfere with insect growth and behaviour [1, 2], we isolated, from neem seeds, several insect growth disrupting substances which do not inhibit feeding in a series of holometabolous insects [3]. The main compound [4] came out to be identical with the well known locust antifeedant azadirachtin [5, 6]. By use of the *Epilachna* bioassay [4], three additional compounds were purified with similar biological and spectroscopic characteristics and we therefore proposed to denote them as a whole group of azadirachtins which were marked with the letters A–D [7]. All the azadirachtins are highly active inhibitors of insect growth and development and they all do not deter insects from feeding.



R=Tigloyl : Azadirachtin B

R=H : Detigloylazadirachtin B

The basic triterpenoid azadirachtin structure is established from NMR [8] and X-ray [9] analyses for the main natural product azadirachtin A, whereas B had been partially characterized only [7]. Independent of our biochemical studies and as such without data about their biological activity, two natural products from neem have been described, both of which having identical NMR spectra. Recently, Klenk *et al.* [10] reported about the structure of 3-tigloyl-azadirachtol and Kubo *et al.* [11] about deacetyl-azadirachtinol. Both these compounds seem to be identical with our bioactive azadirachtin B. However, assignment of the tigloyl moiety to position 3 or alternatively to position 1, can not be given unequivocally by their NMR data. It was desirable therefore to assign the tigloyl moiety by spectral analysis of the detigloyl-azadirachtin B.

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Material and Methods

Azadirachtin B

Crushed neem kernels (60 kg) were agitated in hexane (50 l) and, after filtration, in acetone (30 l). The latter extract was concentrated and then adsorbed on silicic acid (Merck, Darmstadt) by evaporation *in vacuo*. An aliquot of it was placed on a silicic acid column in petrol ether, washed with the same solvent, and the azadirachtin containing material subsequently eluted with petrol ether/ethyl acetate (1:4). This material, after evaporation of the solvent, was further purified by distribution between the two phases formed by a mixture of hexane/methanol/water (5:4:1). The methanol containing lower phase was brought to dryness and further purified by HPLC (RP-8 column, Merck, 300×25 mm). The azadirachtins are eluted with the isocratic solvent mixture methanol/water (7:3). Azadirachtin B partially separates from the main peak containing pure azadirachtin A. Rechromatography of crude azadirachtin B by preparative HPLC (RP-18 column, Latek, Heidelberg, 500×54 mm)

with methanol/water (43:57) as eluent, yields pure azadirachtin B.

Detigloylazadirachtin B

Azadirachtin B (20 mg) was dissolved in methanol (1 ml) and kept for 12 h at room temperature after adding 10% K_2CO_3/water (5 ml). The solution was extracted with chloroform and the product further purified by TLC (Merck SiO_2, 0.5 mm, solvent chloroform/acetone (7:3)). The band at R_f 0.13 yielded, after elution with chloroform, pure detigloylazadirachtin B.

NMR spectroscopy

The ^1H- and ^{13}C NMR spectra were recorded with a Bruker AM-500 spectrometer in $CDCl_3$. Additionally, homonuclear shift – correlated 2D spectra (COSY/Jeener sequence) and 2D-dipolar cross relaxation experiments (NOESY), furthermore 2-D heteronuclear shift correlation (INEPT) were performed.

FAB negative ion mass spectroscopy

Mass spectra were recorded in a Finnigan-Mat 312 instrument with Data System SS 200-MS.

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Results and Discussion

Both azadirachtin B and detigloylazadirachtin B can be obtained with high purity as controlled by analytical HPLC. None of these pure substances could be crystallized, however. The 500 MHz-proton-NMR spectra (Table I) were analyzed by shift calculations, analysis of the spin systems and interpre-

Table I. 500 MHz ^1H NMR data ($CDCl_3$) for azadirachtin B and detigloylazadirachtin B.

	Azadirachtin B shift [ppm], spin state, *J* [Hz]	Detigloylazadirachtin B shift [ppm], spin state, *J* [Hz]	
1-H	3.49 (dt, 5.5, 2.5)	3.61 (dt, 5.5, 3.0)	+0.12
2-Ha	2.29 (dt, 16.2, 2.5)	2.24 (dt, 15.6, 3.0)	−0.05
2-Hb	2.05 (m)	1.81 (dt, 15.6, 3.0)	−0.24
3-H	5.51 (t, 2.5)	4.31 (s_{br})	−1.20
5-H	3.30 (d, 12.6)	3.07 (d, 12.5)	−0.23
6-H	4.52 (dd, 12.6, 2.7)	4.47 (dd, 12.5, 2.6)	−0.05
7-H	4.70 (d, 2.7)	4.69 (d, 2.6)	−0.01
9-H	3.17 (d, < 1)	2.99 (d, < 1)	−0.18
11-H	4.45 (d, < 1)	4.40 (d, < 1)	−0.05
15-H	4.56 (dd, 2.8, < 1)	4.52 (d, 3.6)	−0.04
16-Ha	1.63 (ddd, 5.2, 2.8, 13.1)	1.61 (ddd, 5.2, 3.6, 13.1)	−0.02
16-Hb	1.31 (dt, 13.1, < 1, < 1)	1.30 (d, 13.1)	−0.01
17-H	2.34 (dd, 5.2, < 1)	2.32 (d, 5.2)	−0.02
18-H	2.02 (s)	1.99 (s)	−0.03
19-Ha	3.92 (d, 9.4)	4.07 (d, 9.8)	+0.15
19-Hb	3.47 (d, 9.4)	3.43 (d, 9.8)	−0.04
21-H	5.64 (s)	5.66 (s)	+0.02
22-H	5.01 (d, 2.9)	5.01 (d, 2.9)	0
23-H	6.41 (d, 2.9)	6.40 (d, 2.9)	−0.01
28-Ha	4.01 (d, 9.0)	4.21 (d, 8.6)	+0.20
28-Hb	3.81 (d, 9.0)	4.06 (d, 8.6)	+0.25
30-H	1.42 (s)	1.44 (s)	+0.02
CO_2Me	3.74 (s)	3.76 (s)	+0.02
	3.74 (s)	3.70 (s)	−0.04
3′	6.92 (q, 7.0)	–	
4′	1.77 (d, 7.0)	–	
5′	1.82 (s)	–	
OH	3.38 (d, 5.5)	3.98 (d, 5.5)	
OH	3.23 (C-7 + C-20)	3.85	
OH		3.25	
OH		2.71	

tation of 2D-COSY and NOESY plots. The COSY plots of azadirachtin B confirm the spin analysis as shown in Table I. By use of the INEPT-technique the corresponding carbon and proton resonances of azadirachtin B were assigned. The ^{13}C resonances are in full agreement with the resonances published by Klenk *et al.* [10]. In the NOESY plots the following cross relaxations were observed: 9/18, 5; 30/6, 7, 15, 11, OMe(12); 7/21; 28/5′; 17/22. After saponification the resonances of tiglic acid disappear and most chemical shifts in the spectrum of detigloylazadirachtin B are identical with those of azadirachtin B. However, the spectra of azadirachtin B and of the detigloyl derivative also clearly show differences in the resonance signals of the protons at C-1, C-2b, C-3, C-5, C-9, C-28a, C-28b. The differences in the resonance lines of H-1, H-2b, H-3, H-5, H-9 could be explained with a substitution by tiglic acid either in position C-1, as proposed by Kubo *et al.* [11], or with a substitution at C-3.

Binding of tiglic acid to position 3 becomes clear if the corresponding shift differences of detigloylazadirachtin B (Table I) and of detigloylazadirachtin A (unpublished data) are compared with each other. ΔB/A, 9-H: −0.18/−0.03; 19-Ha: +0.15/+0.04; 28-Ha: +0.20/+0.04; 28-Hb: +0.25/+0.01. In the 2-D-NOESY plot, a cross relaxation of the tiglic acid-methyl-C-5′ with H-28a can be observed which is only possible if tiglic acid is bound at position C-3. The atomic models show that the azadirachtin molecule has an extremely compact structure, even with a hindered rotation of the tigloyl moiety. The FAB negative ion-MS yields a molecular weight of 662 for azadirachtin B and of 580 for detigloylazadirachtin B which again is in accordance with the proposed structures.

[1] H. Rembold, in: Advances in Invertebrate Reproduction 3 (W. Engels *et al.*, eds.), pp. 481–491, Elsevier Science Publishers, Amsterdam 1984.
[2] H. Rembold and E. Winter, in: Proc. Internat. Workshop on Heliothis Management (W. Reed, ed.), pp. 241–250, ICRISAT, Patancheru, A.P. India 1981.
[3] H. Rembold, G. K. Sharma, Ch. Czoppelt, and H. Schmutterer, J. Plant Dis. Prot. **87,** 290–297 (1980).
[4] H. Rembold, G. K. Sharma, Ch. Czoppelt, and H. Schmutterer, Z. ang. Ent. **93,** 12–17 (1982).
[5] J. H. Butterworth and E. D. Morgan, Chem. Commun. **1968,** 23–24.
[6] P. R. Zanno, I. Miura, K. Nakanishi, and D. L. Elder, J. Amer. Chem. Soc. **97,** 1975–1977 (1975).
[7] H. Rembold, H. Forster, Ch. Czoppelt, P. J. Rao, and K.-P. Sieber, in: Natural Pesticides from the Neem Tree (*Azadirachta indica,* A. Juss) and other Tropical Plants (H. Schmutterer and K. R. S. Ascher, eds.), pp. 153–161, GTZ, Eschborn 1984.
[8] W. Kraus, M. Bokel, A. Klenk, and H. Pöhnl, Tetrahedron Lett. **26,** 6435–6438 (1985).
[9] H. B. Broughton, S. V. Ley, A. M. Z. Slawin, D. J. Williams, and E. D. Morgan, J. Chem. Soc., Chem. Commun. **1986,** 46–47.
[10] A. Klenk, M. Bokel, and W. Kraus, J. Chem. Soc., Chem. Commun. **1986,** 523–524.
[11] I. Kubo, I. Matsumoto, and A. Matsumoto, Tetrahedron Lett. **25,** 4729–4732 (1984).