PERGAMON

Phytochemistry 50 (1999) 977–982

PHYTOCHEMISTRY

Insecticidal meliacarpins (C-*seco* limonoids) from *Melia azedarach*

F.I. Bohnenstengel[a], V. Wray[b], L. Witte[c], R.P. Srivastava[d], P. Proksch[a,*]

[a]*Lehrstuhl für Pharmazeutische Biologie, Universität Würzburg, 97082 Würzburg, Germany*
[b]*Gesellschaft für Biotechnologische Forschung mbH, 38124 Braunschweig, Germany*
[c]*Institut für Pharmazeutische Biologie, Universität Braunschweig, 38106 Braunschweig, Germany*
[d]*Department of Entomology, University of Agriculture and Technology, 263145 Pantnagar, India*

Received 11 August 1998

Abstract

Three new meliacarpin derivatives, namely 1,3-dicinnamoyl-11-hydroxymeliacarpin, 1-cinnamoyl-3-methacrylyl-11-hydroxymeliacarpin and 1-cinnamoyl-3-acetyl-11-hydroxymeliacarpin, were isolated as the principal insecticidal and growth disrupting constituents of *Melia azedarach* leaves. Their structures were unambiguously established on the basis of MS spectrometric and NMR spectroscopic data (^{1}H, ^{13}C, COSY, ^{1}H-detected direct and long-range ^{13}C–^{1}H correlations) and by comparison with known compounds. The insecticidal properties of the new meliacarpin derivatives were examined using larvae of the polyphagous pest insect *Spodoptera littoralis*. When incorporated into artificial diet and offered to larvae in a chronic feeding bioassay, 1-cinnamoyl-3-acetyl-11-hydroxymeliacarpin exhibited an EC_{50} of 0.27 ppm and a LC_{50} of 0.48 ppm and is thus comparable with regard to insecticidal activity to the well known natural insecticide azadirachtin. Furthermore, all three meliacarpin derivatives had a pronounced detrimental influence on larval metamorphosis.

Keywords: *Melia azedarach*; Meliaceae; Structure elucidation; Insecticidal activity; C-*seco* limonoid; New meliacarpin derivatives; Azadirachtin; *Spodoptera littoralis*

1. Introduction

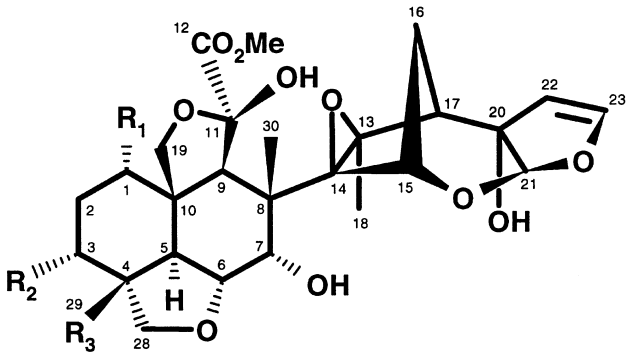
Melia azedarach Linn (Meliaceae), also known as Chinaberry or Persian lilac tree, is a deciduous tree that is native to northwestern India and has long been recognized for its insecticidal properties. In Vietnam leaves of *M. azedarach* are stored between clothes in order to prevent damage by moths. In our continuing search for new insecticidal constituents of plants from the family Meliaceae (Nugroho et al., 1997a, 1997b; Güssregen et al., 1997), we report here the isolation of three new diacylated meliacarpin derivatives isolated from leaves of *M. azedarach* collected in India. All of the isolated compounds showed strong insecticidal activity against larvae of the polyphagous pest insect *Spodoptera littoralis* (Noctuidae).

2. Results and discussion

Crude methanolic extracts from leaves, unripe fruits and stems of *M. azedarach* exhibited significant insecticidal activity when incorporated into artificial diet and tested against neonate larvae of the polyphagous pest insect *S. littoralis* at an arbitrarily chosen concentration of 1300 ppm (data not shown). The methanolic extract of leaves from *M. azedarach* was partitionated between MeOH–hexane, H_2O–CH_2Cl_2 and H_2O–EtOAc, respectively. The CH_2Cl_2 fraction showed strong insecticidal activity. Chromatographic column separation of the CH_2Cl_2 fraction resulted in the isolation of three insecticidal compounds (**1**–**3**) (Fig. 1). Based on their spectral characteristics and on comparison with spectroscopic data of known meliacarpin derivatives such as 1-cinnamoyl-3-feruloyl-11-hydroxymeliacarpin (**4**) (Pöhnl, 1985) and 1,3-dicinnamoyl-22,23-dihydro-11-hydroxy-23β-methoxymeliacarpin (Kaufmann-Horlacher, 1990), they were readily identified as new meliacarpin derivatives. Meliacarpins were found first in *M. azedarach* L. and later in *M. toosendan* Sieb. and Zucc. as well as in the seeds of *Azadirachta indica* A. Juss (Kraus, 1986, 1995; Lee, Klocke, Barnby, Yamasaki, & Ballandrin, 1991; Ascher, Schmutterer, Zebitz, & Naqvi, 1995). The carbon skeleton of the meliacarpins is closely related to those of azadirachtin (**5**). The meliacarpins differ from azadirachtin in that C-29 is not oxidized to a methoxycarbonyl group but is still present as an angular methyl

* Correspondence author. Tel.: +49-931-888-6174; fax: +49-931-888-6182; e-mail: proksch@botanik.uni-wuerzburg.de.

0031-9422/99/$ - see front matter
PII: S0031-9422(98)00644-X



(1)	R_1 = OCin	R_2 = OCin	R_3 = CH_3
(2)	R_1 = OCin	R_2 = $OCOC(CH_3)=CH_2$	R_3 = CH_3
(3)	R_1 = OCin	R_2 = OAc	R_3 = CH_3
(4)	R_1 = OCin	R_2 = OFer	R_3 = CH_3
(5)	R_1 = OTig	R_2 = OAc	R_3 = CO_2CH_3

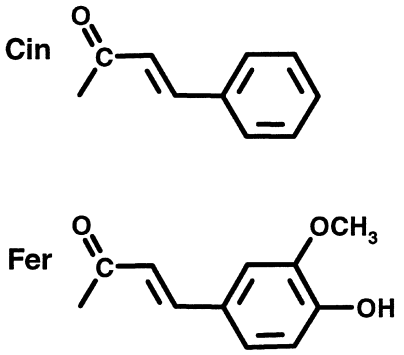


Fig. 1. Structures of new meliacarpin derivatives (**1–3**) isolated in this study (relative configuration shown). Compound (**4**) previously isolated from fruits of *M. azedarach* (Pöhnl, 1985). Compound (**5**): azadirachtin.

group. All new meliacarpins (**1–3**) showed strong UV absorptions in methanol at 279 nm typical of an α,β-unsaturated phenyl group such as that of the *trans*-cinnamoyl group. The identity of the new compounds, and subsequently the assignment of the ^1H and ^{13}C NMR data, followed from the atom connectivities established through 2-D NMR using COSY, ^1H-detected one-bond and multiple-bond ^{13}C–^1H correlation. The meliacarpins have a C-*seco* limonoid skeleton ring and the new natural products (**1–3**) differ from the known compounds with regard to their ester substituents at C-1 and C-3. The ^1H and ^{13}C NMR spectra of the new derivatives were comparable to those of the known 1-cinnamoyl-3-feruloyl-11-hydroxymeliacarpin (**4**) which was previously isolated from the fruits of *M. azedarach* (Pöhnl, 1985). Compound (**4**) is closely related to (**1–3**) except that the feruloyl moiety at C-3 is replaced by a second cinnamoyl moiety in (**1**), a methacryl substituent in (**2**) and an acetyl group in (**3**).

The molecular formula $C_{45}H_{48}O_{14}$, deduced from the molecular ion peak $[M–H]^+$ at m/z 811 in FAB-MS (negative mode) and the $[M+H]^+$ signal at m/z 813 in the ESI-MS (positive mode), was compatible with 1,3-dicinnamoyl-11-hydroxymeliacarpin (**1**). The base peak

at m/z 131 $(C_9H_7O)^+$ in the EI-MS suggested the presence of a cinnamoyl moiety. The presence of a meliacarpin skeleton was concluded from the presence in the ^{1}H NMR spectrum of three methyl singlets at δ 1.11, δ 1.77 and δ 2.21, one methoxyl singlet at δ 3.71 and three hydroxyl singlets at δ 2.84, δ 3.19 and δ 5.03. The presence of two cinnamoyl moieties was evident from the doubling of their characteristic proton and carbon NMR signals. A full assignment of the ^{1}H and ^{13}C data followed from the 2-D spectra (Tables 1 and 2).

1-Cinnamoyl-3-methacrylyl-11-hydroxymeliacarpin (**2**) showed the molecular ion peak $[M+H]^+$ at m/z 751 in the ESI-MS (positive mode) which was compatible with a molecular formula $C_{40}H_{46}O_{14}$. The NMR spectral data of (**2**) were similar to those of compound (**1**) except for the replacement of one cinnamoyl moiety by a methacrylate substituent. The presence of a methacrylate group was indicated by ^{1}H NMR signals at δ 5.39 (dq, 1H), δ 6.02, (b m,1H) and δ 1.79 (bs, 3H) which correlated with the^{13}C NMR signals at δ 166.3 (C-1″), δ 135.9 (C-2″), δ 126.0 (C-3″) and δ 18.2 (C-4″), respectively, and the appropriate long-range correlations in the HMBC spectrum. Unfortunately there were no detectable correlations between the equatorial protons of either H-1 or

Table 1
^{1}H NMR chemical shifts (ppm) and coupling constants (Hz, in parentheses) of meliacarpin derivatives (**1**–**3**) (400 MHz, $CDCl_3$) and (**4**) (250 MHz, $CDCl_3$) (Pöhnl, 1985)

	(**1**)	(**2**)	(**3**)	(**4**)
1	4.76 t (2.5)	4.74 t (2.6)	4.71 t (2.8)	4.73 t (2.5)
2α	2.46 dt (16.8/2.5)	2.44 dt (16.9/2.5)	2.37 dt (16.6/2.8)	2.48 ddd (17.0/2.7/2.5)
2β	2.15 dt (16.8/3.1)	2.15 dt (16.9/3.2)	2.09 dt (16.6/3.1)	2.13 ddd (17.0/2.7/2.5)
3	5.04 t (3.1)	4.96 t (2.9)	4.92 t (2.8)	5.00 t (2.7)
5	3.28 d (12.7)	3.18 d (12.7)	3.16 d (12.7)	3.29 d (12.6)
6	4.21 dd (12.7/2.6)	4.19 dd (12.7/2.7)	4.19 dd (12.7/2.7)	4.22 dd (12.6/2.8)
7	4.74 d (2.6)	4.72 d (2.7)	4.75 d (2.6)	4.78 d (2.8)
9	3.46 s	3.41 s	3.41 s	3.45 s
15	4.67 d (3.5)	4.66 d (3.5)	4.66 d (3.6)	4.68 d (3.3)
16α	1.71 ddd (13.1/5.3/3.7)	1.70 ddd (13.3/5.3/3.7)	1.70 ddd (13.1/5.4/3.8)	1.72 ddd (13.1/5.0/3.3)
16β	1.33 d (13.1)	1.34 d (13.3)	1.33 d (13.1)	1.34 d (13.1)
17	2.40 d (5.2)	2.40 d (5.3)	2.39 d (5.4)	2.42 d (5.0)
18	2.21 s	2.20 s	2.20 s	2.22 s
19a	3.87 d (9.5)	3.85 d (9.4)	3.83 d (9.5)	3.87 d (9.5)
19b	4.24 d (9.5)	4.22 d (9.4)	4.22 d (9.5)	4.24 d (9.5)
21	5.72 s	5.70 s	5.71 s	5.74 s
22	5.04 d (2.9)	5.03 d (3.0)	5.03 d (2.9)	5.05 d (2.9)
23	6.45 d (2.8)	6.44 d (3.0)	6.45 d (3.0)	6.46 d (2.9)
28α	3.75 d (8.3)	3.71 d (8.2)	3.73 d (8.2)	3.80 d (8.2)
28β	3.69 d (8.3)	3.66 d (8.2)	3.66 d (8.2)	3.70 d (8.2)
29	1.11 s	1.09 s	1.06 s	1.10 s
30	1.77 s	1.76 s	1.76 s	1.77 s
7-OH	2.84 s	2.77 s	2.76 s	2.98 s
11-OH	5.03 s	4.97 s	4.99 s	5.06 s
20-OH	3.19 b s	3.32 b s	3.17 s	3.29 s
12-OMe	3.71 s	3.69 s	3.71 s	3.70 s
Cinnamoyl				
ph′-H	7.00–7.40 m (5H)	7.37–7.45 m (5H)	7.38–7.51 m (5H)	7.22–7.39 m (5H)
ph″-H	7.00–7.40 m (5H)			
7′	7.74 d (16.0)	7.66 d (15.9)	7.72 d (16.0)	7.69 d (16.0)
7″	7.57 d (16.0)			
8′	6.36 d (15.9)	6.27 d (15.9)	6.35 d (16.0)	6.31 d (16.0)
8″	6.17 d (16.0)			
Methacrylate				
3″		5.39 dq (1.5), 6.02 b m		
4″		1.79 b s		
CH_3COO			1.88 s	

^{1}H NMR data of compound (**4**) (Pöhnl, 1985) are included for comparison (without chemical shifts of the feruloyl group).

Table 2
^{13}CNMR spectral data of compounds (**1**–**2**) (100 Mhz, $CDCl_3$) and (**4**) (62,89 Mhz, $CDCl_3$) (Pöhnl, 1985)

C	(**1**)	(**2**)	(**4**)
1	71.06 d*	70.98 d	71.03 d
2	27.98 t	28.04 t	27.76 t
3	70.96 d*	71.25 d	70.87 d
4	42.82 s	42.85 s	42.74 s
5	35.69 d	35.81 d	35.44 d
6	72.72 d	72.71 d	72.97 d
7	75.39 d	75.34 d	75.21 d
8	44.97 s+	44.86 s+	45.12 s
9	44.83 d+	44.78 d+	44.72 d
10	50.12 s	50.02 s	50.12 s
11	104.11 s	104.12 s	104.08 s
12	171.86 s	171.78 s	171.87 s
13	68.83 s	68.71 s	68.92 s
14	70.03 s	70.03 s	70.07 s
15	76.29 d	76.32 d	76.19 d
16	25.18 t	25.06 t	25.28 t
17	49.00 d	49.11 d	48.84 d
18	18.33 q	18.28 q	18.32 q
19	69.92 t	69.93 t	69.87 t
20	83.71 s	83.68 s	83.62 s
21	109.05 d	109.07 d	108.81 d
22	107.39 d	107.35 d	107.54 d
23	147.00 d	146.93 d	146.83 d
28	77.19 t	77.17 t	77.85 t
29	19.00 q	19.06 q	18.81 q
30	21.33 q	21.30 q	21.36 q
12-OMe	53.33 q	53.34 q	53.12 q
Cinnamoyl			
1′	133.76 s	134.14 s	133.9 s
2′/6′	128.00 d	127.98 d	127.97 d
3′/5′	128.79 d	129.08 d	128.91 d
4′	130.37 d	130.60 d	130.45 d
7′	145.66 d	145.60 d	145.56 d
8′	117.20 d	117.27 d	117.62 d
9′	165.47 s	165.73 s	165.59 s
1″	134.03 s		
2″/6″	128.14 d		
3″/5″	129.11 d		
4″	130.57 d		
7″	145.95 d		
8″	117.69 d		
9″	166.00 d		
Methacrylate			
1″		166.34 s	
2″		135.93 s	
3″		126.01 t	
4″		18.20 q	

*, + Assignments in each column may be interchanged.
^{13}C NMR data of compound (**4**) (Pöhnl, 1985) are included for comparison (without chemical shifts of the feruloyl group).

H-3 to the respective carbonyl carbons of the substituents in this spectrum. Hence the relative magnitudes of the shifts in the ^{1}H spectrum relative to (**1**) have been used to establish the position of the methacrylate group. Thus the upfield of 0.08 ppm of H-3 compared to 0.02 ppm of H-1 has been taken as an indication that replacement of the bulky cinnamoyl group by a methacrylate substituent has taken place at C-3.

1-Cinnamoyl-3-acetyl-11-hydroxymeliacarpin (**3**) was compatible with the molecular formula $C_{38}H_{44}O_{14}$ deduced from the molecular ion peak $[M\text{-}H]^+$ at m/z 723 in the FAB-MS (negative mode) and from the $[M+H]^+$ peak at m/z 725 in the ESI-MS (positive mode). The ^{1}H NMR spectral data of (**3**) were also comparable to those of (**1**). The presence of an acetyl substituent at C-3 was evident from the singlet at δ 1.88 in the ^{1}H NMR spectrum. An upfield shift for H-3 caused by the replacement of the bulky cinnamoyl group of compound (**1**) with the small acetyl group supported this assignment. The small amount of compound (**3**) did not allow ^{13}C NMR spectra to be recorded.

Meliacarpins (**1**–**3**) and azadirachtin (**5**) were evaluated for their insecticidal activity against neonate larvae of the polyphagous pest *S. littoralis*. Their LC_{50} values and EC_{50} values are given in Table 3. Of the new compounds isolated, 1-cinnamoyl-3-acetyl-11-hydroxymeliacarpin (**3**) was the most active derivative. Its LC_{50} and EC_{50} values (LC_{50} 0.48 ppm and EC_{50} 0.27 ppm) are comparable to those of azadirachtin (LC_{50} 0.32 ppm and EC_{50} 0.11 ppm).

It is obvious that the nature of the ester substituent at C-3 plays an important role for the insecticidal activity of the new compounds. The most active compound (**3**) contained a small and relatively hydrophilic acetyl group at C-3, whereas compound (**1**) with a bulky and more lipophilic cinnamoyl group exhibited the weakest activity. These results confirm previous findings on quantitative structure activity relationships of azadirachtin derivatives, namely that the insecticidal activity of the

Table 3
LC_{50} and EC_{50} values of insecticidal meliacarpin derivatives (**1**–**3**) and of azadirachtin towards neonate larvae of *S. littoralis*

Compound	LC_{50} (ppm)	EC_{50} (ppm)
(**1**)	2.36 ± 0.77	0.57 ± 0.44
(**2**)	1.19 ± 0.35	0.57 ± 0.30
(**3**)	0.48 ± 0.21	0.27 ± 0.19
Azadirachtin	0.32 ± 0.13	0.11 ± 0.08

Data are mean values from triplicates ($\pm$SD).
Chronic feeding experiments: neonate larvae of *S. littoralis* ($n=20$) were released on diet spiked with various concentrations of the analyzed compounds (0.01–6.0 ppm). After six days of exposure, the surviving larvae were weighed to obtain EC_{50} values (concentration inhibiting larvae growth by 50% relative to controls), transferred to fresh untreated diet and reassessed after another 6 days. The survival rate of larvae after these 12 days was recorded and compared to controls that had been exposed to diet treated with solvent methanol. From the respective dose–response curves LC_{50} and EC_{50} values were calculated by probit analysis.

respective natural products depends on the polarity of ring A (Rembold & Puhlmann, 1995) and on the size of the ester substituents (Hansen, Cuomo, Mamunur, Gallagher, & Ellenberger, 1992).

3. Experimental

3.1. Isolation and spectroscopic identification of compounds

Leaves of *M. azedarach* L were collected in India (near Pantnagar) in June 1996. Voucher specimens are on file in the University of Agriculture and Technology in Pantnagar (RPS). Air dried leaves of *M. azedarach* (162 g dry wt.) were ground and extracted successively with Me_2CO and MeOH. The total extract was evaporated under reduced pressure and partitionated between MeOH–hexane, H_2O–CH_2Cl_2 and H_2O–EtOAc. Each fr. was submitted to a bioassay with neonate larvae (see below). From this bioassay, the insecticidal activity was found to reside in the CH_2Cl_2 and EtOAc-fr. Bioassay guided fractionation of the CH_2Cl_2-fr. was achieved through repeated column chromatography sepn. employing silica gel (Merck, Darmstadt, FRG) (mobile phase: CH_2Cl_2–*iso*-propanol 93:7) and Sephadex LH-20 (Sigma, Deisenhofen, FRG) (mobile phase: Me_2CO). Final purification was obtained using RP-18 lobar columns (Merck, Darmstadt, FRG) (mobile phase: mixts. of MeOH and H_2O) and by preparative HPLC. The separation column (250×8 mm, i.d.) was prefilled with Eurospher RP-18, (Knauer, Berlin, FRG). Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. Frs. were monitored by TLC on precoated TLC plates with Si gel 60 F_{254} (Merck, Darmstadt, FRG) (mobile phase: CH_2Cl_2–*iso*-propanol 93:7). Cinnamoyl substituted meliacarpin derivatives were detected by their dark absorbance under UV_{254} nm and by spraying the TLC plates with anisaldehyde reagent. Yields of compounds (**1**–**3**) were: (**1**) 3.89 mg, (**2**) 4.66 mg and (**3**) 0.78 mg.

UV spectra were measured with a Perkin Elmer UV–VIS Spectrometer Lambda 2. ^{1}H NMR (1-D and 2-D COSY), ^{13}C NMR (1-D, DEPT-135) and 2-D ^{1}H-detected one-bond (HMQC) (Bax & Subramanian, 1986) and multiple-bond (HMBC) (Bax & Summers, 1986) ^{13}C–^{1}H correlations were recorded on a Bruker ARX 400 NMR spectrometer. FAB-MS (glycerine as matrix) and EI-MS spectra were recorded on a Finnigan MAT 8430 mass spectrometer. ESI-MS were recorded on a Finnigan MAT TSQ-7000 triple stage quadrupole mass spectrometer. The temperature of the heated capillary (20 V) was 220°C and the electrospray capillary voltage was set to 3.5 kV. Nitrogen served both as sheat (70 psi) and auxiliary gas; argon served as collision gas. Optical rotations were determined on a Perkin Elmer 241 MC.

3.2. Experiments with insects

The chronic feeding assays were carried out with larvae of the polyphagous pest insect *S. littoralis* (Noctuidae, Lepidoptera). *S. littoralis*, the so-called Egyptian cotton worm, is a notorious pest insect in North Africa. The larvae were from a laboratory colony reared on artificial diet under controlled conditions at 26° as described previously (Srivastava & Proksch, 1991; Nugroho et al., 1998). The neonate larvae were forced to feed on a diet treated with various concentrations of the test compounds (0.01, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.8, 2.0, 3.0, 4.0 and 6.0 ppm). After a 6-day exposure, the larvae were weighed (for calculation of the EC_{50} value) transferred to a fresh untreated diet and reassessed after another 6 days. The number of surviving larvae were recorded after 12 days and compared to controls that had been exposed to diet treated with pure methanol only. All experiments were carried out in triplicate. The LC_{50} and EC_{50} values of the meliacarpin derivatives were calculated from the dose-response curves by probit analysis. Azadirachtin, which was used as a positive control, was obtained from Roth (Karlsruhe, F.R.G.).

3.2.1. 1,3-Dicinnamoyl-11-hydroxymeliacarpin (*1*)

White amorphous residue, $C_{45}H_{48}O_{14}$: λ^{MeOH}_{max} nm ($\log\varepsilon$): 217.4 (4.44), 273.0 (4.54); $[\alpha]^{20}_D$ +36.8° ($CHCl_3$; $c = 1.0$); FAB-MS (glycerol as matrix): 811 $[M–H]^+$; ESI-MS m/z: 813 $[M+H]^+$(2), 795 $[M–H_20]^+$ (100), EI-MS (70 eV) m/z (rel. init.): 752 $[M–HOAc]^+$ (1), 651 $[M-161]^+$ (1), 539 (10), 195 (16), 151 (6), 131 $[Cin]^+$ (100), 95 (10).

3.2.2. 1-Cinnamoyl-3-methacrylyl-11-hydroxymeliacarpin (*2*)

White amorphous residue, $C_{40}H_{46}O_{14}$: λ^{MeOH}_{max} nm ($\log\varepsilon$): 215.4 (4.21), 279.2 (4.13); $[\alpha]^{20}_D$ +17.6° ($CHCl_3$; $c = 1.0$); ESI-MS m/z: 751 $[M+H]^+$ (3), 733 $[M–H_20]^+$ (100); EI-MS (70 eV) m/z (rel. init.): 690 $[M–HOAc]^+$ (1), 618 $[M–Cin]^+$ (1), 589 $[M-161]^+$ (5), 195 (12), 151 (18), 131 $[Cin]^+$ (100), 95 (26).

3.2.3. 1-Cinnamoyl-3-acetyl-11-hydroxymeliacarpin (*3*)

White amorphous residue, $C_{38}H_{44}O_{14}$: λ^{MeOH}_{max} nm ($\log\varepsilon$): 216.2 (3.89), 279.0 (3.87); $[\alpha]^{20}_D$ +21.0° ($CHCl_3$; $c = 1.0$); FAB-MS (glycerol as matrix): 723 $[M–H]^+$; ESI-MS m/z: 725 $[M+H]^+$ (2), 707 $[M–H_20]^+$ (100); EI-MS (70 eV) m/z (rel. init.): 664 $[M–HOAc]^+$ (4), 592 $[M–Cin]^+$ (1), 563 $[M-161]^+$ (18), 195 (22), 151 (24), 131 $[Cin]^+$ (100), 95 (42).

Acknowledgements

We would like to thank Dr. Herderich (University of Würzburg) for the opportunity to carry out ESI-MS measurements. Furthermore we would like to thank Dr.

R. A. Edrada (University of Würzburg) for helpful discussions and B. Jaschok-Kentner (GBF) for recording the NMR data. RPS thanks the DAAD for a scholarship. Financial support by a grant of the BMBF/Bayer AG is gratefully acknowledged.

References

Ascher, K. R. S., Schmutterer, H., Zebitz, C. P. W., & Naqvi, S. N. H. (1995). In H. Schmutterer (Ed.), *The neem tree Azadirachta indica A. Juss. and other meliaceous plants* (p. 605). Weinheim, Germany: VCH.

Bax, A., & Subramanian, S. (1986). *Journal of Magnetic Resonance*, *67*, 565–569.

Bax, A., & Summers, M. F. (1986). *Journal of the American Chemical Society*, *108*, 2093–2094.

Güssregen, B., Fuhr, M., Nugroho, B. W., Wray, V., Witte, L., & Proksch, P. (1997). *Zeitschrift fuer Naturforschung*, *52c*, 339.

Hansen, D. J., Cuomo, J., Mamunur, K., Gallagher, R. T., & Ellenberger, W. P. (1992). In P. A. Hedin, J. J. Menn, & R. M. Hollingworth (Eds.), *Natural and engineered pest management agents* (Vol. 551, p. 103). Washington DC: ACS Symp. Ser.

Kaufmann-Horlacher, I. (1990). Unpublished doctoral dissertation, University Hohenheim, Germany.

Kraus, W. (1986). *Studies in Organic Chemistry*, *26*, 237.

Kraus, W. (1995). In H. Schmutterer (Ed.), *The neem tree Azadirachta indica A. Juss. and other meliaceous plants* (p. 35). Weinheim, Germany: VCH.

Lee, S. M., Klocke, J. A., Barnby, M. A., Yamasaki, R. B., & Ballandrin, M. F. (1991). In P. A. Hedin (Ed.), *Naturally occuring pest bioregulators* (Vol. 449, p. 293). Washington, DC: ACS Symp. Ser.

Nugroho, B. W., Edrada, R. A., Bohnenstengel, F., Supriyono. A., Eder, C., Handayani, D., & Proksch, P. (1998). In P. Schreier, M. Herderich, H.-U. Humpf, & W. Schwab (Eds.), *Natural product analysis* (p. 373). Wiesbaden: Vieweg.

Nugroho, B. W., Edrada, R. A., Güssregen, B., Wray, V., Witte, L., & Proksch, P. (1997). *Phytochemistry*, *44*, 1455.

Nugroho, B. W., Güssregen, B., Wray, V., Witte, L., Bringmann, G., & Proksch, P. (1997). *Phytochemistry*, *45*, 1579.

Pöhnl, H. (1985). Unpublished doctoral dissertation, University Hohenheim, Germany.

Rembold, H., & Puhlmann, I. (1995). In H. Schmutterer (Ed.), *The neem tree Azadirachta indica A. Juss. and other meliaceous plants* (p. 222). Weinheim, Germany: VCH.

Srivastava, R. P., & Proksch, P. (1991). *Entomology and Generics*, *15*, 265.