New Insecticidal Rocaglamide Derivatives from Flowers of *Aglaia odorata*

B. Güssregen[a], M. Fuhr[a], B. W. Nugroho[a,*], V. Wray[b], L. Witte[c] and P. Proksch[a]

[a] Julius-von-Sachs-Institut für Biowissenschaften, Lehrstuhl für Pharmazeutische Biologie, Universität Würzburg, Mittlerer Dallenbergweg 64, D-97082 Würzburg, Fax: 0049 931 888 6182, Bundesrepublik Deutschland

[b] Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124 Braunschweig,Bundesrepublik Deutschland

[c] Institut für Pharmazeutische Biologie, Technische Universität Braunschweig, Mendelssohnstr. 1, D-38106 Braunschweig, Bundesrepublik Deutschland

Z. Naturforsch. **52c,** 339–344 (1997); received December 30, 1996/February 13, 1997

Aglaia odorata, Meliaceae, Rocaglamide Derivatives, Structure Elucidation, Natural Insecticides

Flowers of *A. odorata* yielded six insecticidal rocaglamide derivatives including four compounds which proved to be new natural products. Structure elucidation of the new compounds, including establishment of their absolute configurations by CD spectroscopy and NMR is described. When incorporated into artificial diet all isolated rocaglamide derivatives exhibited strong insecticidal activity towards neonate larvae of the polyphagous pest insect *Spodoptera littoralis* with LC_{50}s varying from 1.5–53.4 ppm (2.9–97.1 nM). Two of the isolated compounds which showed LC_{50}s of 1.5 and 1.6 ppm (2.9 and 3.2 nM) respectively are similar with regard to their insecticidal activity to azadirachtin (LC_{50} 0.9 ppm [1.3 nM]) which was included into the study as a positive control.

Introduction

The family Meliaceae is the source of numerous insecticidal compounds with azadirachtin isolated from the Neem tree (*Azadirachta indica*) being the most familiar example (Rembold, 1989; Klocke, 1989). Recently, members of the genus *Aglaia* Lour. such as *A. odorata* and *A. duperreana* were shown to contain novel insecticidal constituents of the rocaglamide type that feature a cyclopentatetrahydrobenzofuran skeleton (Janprasert *et al.*, 1993; Ishibashi *et al.*, 1993; Nugroho *et al.*, 1997). The insecticidal activity reported for rocaglamide, as well as for several of its congeners, prompted us to screen for further insecticidal rocaglamide derivatives from the genus *Aglaia*. In this study we report the identification of six insecticidal rocaglamide derivatives, including four new natural products from flowers of *A. odorata* Lour.

* Permanent address: Department of Plant Pests and Diseases, Faculty of Agriculture, Bogor Agricultural University, Jl. Raya Pajajaran-Bogor 16144, Indonesia.

Reprint requests to Prof. Proksch.

Materials and Methods

Plant material

Flowers of *A. odorata* were collected in December of 1994 on the island of Java (Indonesia). Exact localities can be obtained from the authors. A voucher specimen is on file in the J.-v.-Sachs-Institut für Biowissenschaften, Univ. Würzburg.

Extraction and isolation

Air dried flowers (370 g dry wt) were powdered and exhaustively extracted with MeOH. Following evaporation of the solvent the extract was partitioned between H_2O/hexane, H_2O/CH_2Cl_2, H_2O/EtOAc and H_2O/n-butanol. Each fraction obtained was submitted to a bioassay with neonate larvae (see below). In this bioassay the insecticidal activity was found to reside in the CH_2Cl_2 fraction. Bioassay-guided fractionation of this fraction was achieved through repeated chromatographic separation employing silica gel (Merck, Darmstadt, FRG) (mobile phase: CH_2Cl_2/iso-propanol 90:10 v/v) and Sephadex LH-20 (Sigma, Deisenhofen, FRG) (mobile phase: Me_2CO). Final purification was achieved using RP-18 lobar columns (Merck, Darmstadt, FRG) (mobile phase: 70% aq.

0939–5075/97/0500–0339 $ 06.00

MeOH). Fractions were monitored by TLC on premade silica gel plates (F_{254}) (Merck, Darmstadt, FRG) (mobile phase: CH_2Cl_2/iso-propanol 90 : 10 v/v). Rocaglamide derivatives were detected by their dark absorbance under UV_{254} nm or after spraying with the anisaldehyde reagent. Yields were: **1:** 12.7 mg, **2:** 8.6 mg, **3:** 4 mg, **4:** 6.8 mg, **5:** 1.4 mg, **6:** 3.0 mg.

^1H and ^{13}C NMR spectra were recorded on Bruker AM 300 or ARX 400 NMR spectrometers. EI-MS spectra (70 eV) were obtained by direct inlet. CD spectra were recorded in ethanol. The structures of **1** – **6** were obtained from 1D (^1H and ^{13}C) and 2D (COSY, ^1H-detected direct, and long-range ^{13}C-^1H correlations) spectra and by comparison with published data (Janprasert *et al.*, 1993; Ishibashi *et al.*,1993; Nugroho *et al.*, 1997; Trost *et al.*, 1990; Kokpol *et al.*, 1994; Dumontet *et al.*, 1996).

3: $[a]_D^{20}$ -44.9 (c = 0.12, $CHCl_3$). CD: 217 nm ($\Delta\varepsilon$ -17), 228 nm ($\Delta\varepsilon$ -8). EI-MS (*m/z*, rel. int.): 549 (16) $(M)^+$, 531 (16), 489 (75), 458 (40), 431 (52), 329 (100), 316 (84), 301 (48), 283 (54), 181 (82), 162 (25), 151(18), 42(25).

4: $[a]_D^{20}$ -44.1 (c = 0.22, $CHCl_3$). CD: 218 nm ($\Delta\varepsilon$ -17), 223 nm ($\Delta\varepsilon$ -15), 230 nm (sh) ($\Delta\varepsilon$ -10). EI-MS (*m/z*, rel. int.): 493 (5) $(M)^+$, 475 (15), 458 (12), 433 (30),432 (100), 340(20), 327(26), 317(32), 300(52), 285(24), 181 (15), 148 (10).

5: $[a]_D^{20}$ -56.3 (c = 0.12, $CHCl_3$). CD: 217 nm ($\Delta\varepsilon$ -18), 228 nm (sh) ($\Delta\varepsilon$ -12). EI-MS (*m/z*, rel. int.): 550 (20) $(M)^+$, 490 (20), 431 (10), 336(10), 329 (34), 316 (60), 301 (34), 283 (100), 181 (50).

6: $[a]_D^{20}$ -109.3 (c = 0.20, $CHCl_3$). CD: 218 nm ($\Delta\varepsilon$ -25). EI-MS (*m/z*, rel. int.): 464 (20), 446 (5), 343 (8), 330 (100), 315 (20), 181 (8), 164(10).

7: $[\alpha]_D^{20}$ -89.0 (c = 0.17, $CHCl_3$). CD: 217 nm ($\Delta\varepsilon$ -17). EI-MS (*m/z*, rel. int.): 505 $(M)^+$ (19), 487 (8), 390 (40), 313 (59), 300 (57), 285 (37), 205 (26), 181 (40), 176 (100), 135 (18), 131 (18).

Experiments with insects

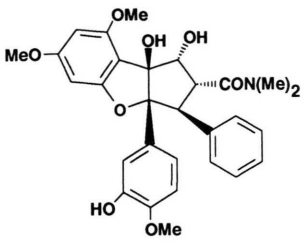
Larvae of *S. littoralis* were from a laboratory colony reared on artificial diet under controlled conditions as described previously (Srivastava *et al.*, 1990). Feeding studies were conducted with neonate larvae (n = 20 for each treatment) that were kept on diet treated with extracts or compounds under study. After 6 days survival and weight of the surviving larvae were recorded and compared with controls. LC_{50}s and EC_{50}s were calculated from dose-response curves by probit-analysis. Azadirachtin, which was used as a positive control, was commercially available from Roth (Karlsruhe, FRG).

Results and Discussion

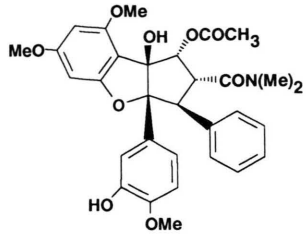
Extraction of air dried flowers of *A. odorata* with MeOH yielded a crude extract with powerful insecticidal activity towards neonate larvae of the polyphagous plant pest *Spodoptera littoralis* (Noctuidae), which was used as a model to screen for insecticidal activity. When the extract was incorporated into artificial diet at an arbitrarily chosen concentration of 1000 ppm diet and offered to larvae in a chronic feeding bioassay none of the insects was found to survive beyond the first 2–3 days of the experiment (data not shown). Repeated bioassay-guided separation of the extract resulted in the isolation of 6 insecticidal compounds (**1** – **6**, Fig. 1) which were readily identified as derivatives of rocaglamide (**7**) based on their spectral characteristics.

Compounds **1** and **2** were recently described from stems of *Aglaia duperreana* collected in Vietnam (Nugroho *et al.*, 1997). Compounds **3** – **6** are new natural products differing from the known compounds **1** and **2** as well as from each other with regard to their substituents at C-1 and/or at C-2 (and at C-3' in the case of compound **6**). Compound **3** is a new desmethyl derivative of **2** as evident from its molecular weight of 549 which is 14 mass units lower than that of **2**. Inspection of the ^1H and ^{13}C NMR spectra of **3** (Table I) indicates only one methyl substituent in the aminoacyl side chain (three proton doublet at 2.58 ppm through coupling to NH which is not exchanged in acetone as solvent, carbon signal at 26.1 ppm) of **3** compared to two methyl groups in compound **2**.

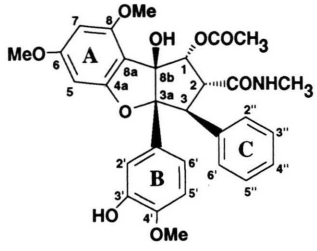
Compound **4** differs from **3** by loss of the acetyl substituent esterified at C-1 as evident from the upfield shift of H-1 (4.72 ppm) in the ^1H NMR spectrum of **4** compared to the H-1 signal (6.00 ppm) of **3** (Table I), as well as from loss of acetyl carbon signals in the ^{13}C NMR spectrum of **4** (Table I). A further difference between **4** and **3** lies in the nature of their aminoacyl substituents. The $CONHCH_3$ group of **3** is replaced by a



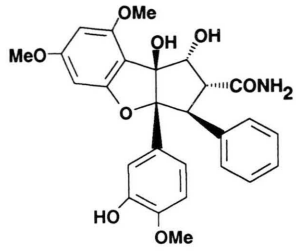
1



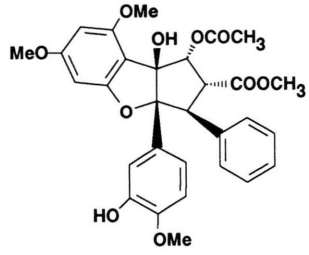
2



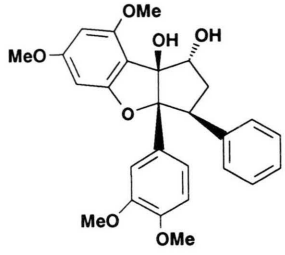
3



4

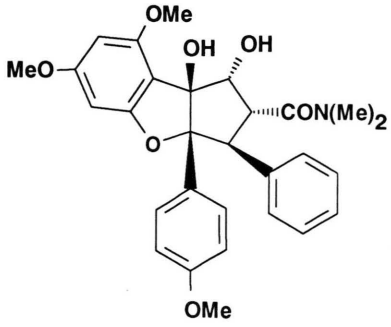


5



6

Fig. 1. Structures of rocaglamide derivatives (**1–6**) isolated from flowers of *Aglaia odorata* and of rocaglamide (**7**) previously isolated from leaves of *A. odorata*.



7

$CONH_2$ substituent as indicated by the molecular weight of **4** and by comparison of the ^1H and ^{13}C NMR spectra of **4** and **3** (proton and carbon signals corresponding to the $NH\underline{CH_3}$-substituent of **3** are absent in the spectra of **4**) (Table I).

Compound **5** is a new derivative of methylrocaglate that was previously isolated from leaves of *A. odorata* (Janprasert *et al.*, 1993). The new compound differs from the known methylrocaglate by the presence of an O-acetyl, instead of an OH-substituent, at C-1 as well as by an additional OH-group in ring B. Inspection of the mass spectrum of **5** reveals the characteristic loss of 60 mass units corresponding to acetic acid. The ^1H NMR spectrum of **5** shows the typical downfield shift of

Table I. ^{1}H and ^{13}C NMR data of compounds **3–6** isolated from flowers of *A. odorata* and of rocaglamide (**7**).

^{1}H NMR	**3** (in d-acetone)	**4** (in d-acetone)	**5** (in d-MeOH)	**6** (in d-MeOH)	**7** (in d-MeOH)
1	6.00 (d, 5.9)	4.72 (d, 5.1)	6.05 (d, 5.3)	4.76 (bd, 5.9)	5.01 (d, 6.8)
2A				2.81 (ddd, 13.9, 13.7, 6.1)	
B	4.02 (dd, 5.8, 14.3)	3.98 (dd, 5.2, 14.1)	4.12 (dd, 5.3, 14.4)	2.13 (ddd, 13.4, 6.3, 1.1)	4.17 (dd, 6.8, 13.6)
3	4.28 (d, 14.3)	4.39 (d, 14.1)	4.18 (d, 14.5)	3.98 (dd, 6.4, 14.0)	4.41 (d, 13.6)
5	6.26 (d, 1.9)	6.22 (d, 1.9)	6.31 (d, 1.9)	6.34 (d, 1.9)	6.35 (d, 1.9)
7	6.11 (d, 2.0)	6.12 (d, 2.0)	6.17 (d, 1.8)	6.24 (d, 1.9)	6.23 (d, 2.0)
2′	6.81 (d, 2.2)	6.77 (d, 2.1)	6.79 (d, 2.1)	6.66 (d, 2.0)	7.17 (m)
3′					6.69 (d, 9.0)
5′	6.60 (d, 8.6)	6.58 (d, 8.6)	6.68 (d, 8.6)	6.76 (8.5)	6.69 (d, 9.0)
6′	6.69 (dd, 2.4, 8.5)	6.67 (dd, 2.3, 8.5)	6.75 (dd, 2.1, 8.5)	6.93 (dd, 2.1, 8.4)	7.17 (m)
2″, 6″	7.03 (m)	7.15 (d, 7.1)	6.99 (m)	7.04 (m)	6.91 (m)
3″, 5″	7.03 (m)	7.04 (dd, 7.2, 8.0)	7.08 (m)	7.11 (m)	7.05 (m)
4″	6.98 (m)	6.98 (dd, 7.2, 7.2)	7.08 (m)	7.11 (m)	7.05 (m)
OMe-6	3.84 (s)	3.81 (s)	3.87 (s)	3.87 (s)	3.87 (s)
OMe-8	3.73 (s)	3.83 (s)	3.79 (s)	3.91 (s)	3.89 (s)
OMe-3′				3.58 (s)	
OMe-4′	3.70 (s)	3.68 (s)	3.76 (s)	3.76 (s)	3.71 (s)
$N\underline{CH_3}$	2.58 (d, 4.6)*				2.92 (s); 3.39 (s)
$O\underline{C}OMe$	1.76 (s)		1.86 (s)		
$CO\underline{OMe}$			3.61 (s)		

* Doublet becaused coupling to NH which is not exchanged in acetone as solvent.

^{13}C NMR	**3** (in d-acetone)	**4** (in d-acetone)	**5** (in d-MeOH)	**6** (in d-MeOH)	**7** (in d-MeOH)
1	80.1	80.9	80.4	80.1	79.8
2	51.2	51.4	52.5	37.6	37.4
3	56.9	55.8	57.8	54.7	57
3a	102.4	102.6	102.3	104.2	102.6
5	89.1	89.3	89.5	90.1	90.3
7	92.4	92.5	92.8	93.1	93.2
8a	108.8	109.2	108.5	109.5	109.5
8b	93.5	94.8	93.9	95.9	95.2
1′	130.0	130.4	129.8	130.3	129.4
2′	116.3	116.3	116.6	114.3	130.2
3′	145.8	145.7	146.1	148.9	113.3
5′	110.6	110.5	111.2	111.3	113.3
6′	120.2	120.1	120.7	121.9	130.2
1″	139.2	139.4	138.4	140.7	139.8
2″, 6″	128.9	129.3	129.0	129.3	129.1
3″, 5″	128.1	128.1	128.6	128.6	128.4
4″	126.8	126.7	127.5	127.2	127.1
4′, 8, 4a, 6	146.9	146.8	147.9	149.3	159.8
	159.4	159.2	159.7	159.2	159.2
	161.4	162.3	161.9	162.2	162.2
	164.6	164.3	165.4	165.2	165.3
$Ar\underline{OMe}$	56.0, 55.9, 55.6	57.1, 56.0, 55.8	56.2, 56.1, 55.8	54.7, 56.1, 56.3, 56.4	56.1, 56.1, 55.5
$\underline{C}=O$	169.3	173.9	171.8		171.7
$O\underline{C}OCH_3$	169.0		170.9		
$N-\underline{CH_3}$	26.1				36.0, 30.7
$CO\underline{OMe}$			51.0		
$OCO\underline{CH_3}$	20.9		20.8		

H-1 (6.05 ppm) indicative of an O-acetyl residue at C-1 (Table I). Assignment of the OH-substituent at C-3' is based on the coupling pattern of the neighbouring aromatic ring protons (Table I).

Compound **6** is a new derivative of rocaglaol that was previously isolated from leaves of *A. odorata* (Janprasert *et al.*, 1993). The new compound (**6**) differs from rocaglaol by the presence of an additional methoxyl group at C-3' of ring B. Assignment of the respective OCH_3 group is based on NOE experiments. Irradiation at the methoxy substituent at C-3' causes an enhancement

of the signal of H-2', whereas irradiation at the methoxy group at C-4' results in an enhancement of the signal of H-5'.

Compound **1** – **6** are closely related to rocaglamide (**7**) that was previously isolated from leaves of *A. odorata* (Janprasert *et al.*, 1993; Ishibashi *et al.*, 1993) and from stems of *A. duperreana* (Nugroho *et al.*, 1997). The absolute configuration of rocaglamide is known through enantioselective synthesis (Trost *et al.*, 1990). The CD spectrum of rocaglamide (**7**) was recently published (Nugroho *et al.*, 1997). Since compounds **1** – **6** were isolated from the same species as **7** they should have the same absolute configuration as rocaglamide (**7**). This assumption is corroborated by inspection of the CD and ^{1}H NMR spectra of **1** – **7**. All CD spectra are very similar showing prominent negative Cotton effects around 218 nm as most characteristic features. Hence, it seems feasible that compounds **1** – **7** should have the same absolute configuration at the asymmetric carbons C-3, C-3a, C-8b and possibly C-1 which are adjacent to the three aromatic rings that form the major molecular chromophors of the cyclopentatetrahydrobenzofuran moieties. The asymmetric carbon C-2, however, has no influence on the CD spectra since compound **6** which lacks the stereocenter at C-2 has virtually the same CD spectrum as compounds **1** – **5** and **7**. Assignment of the configurations at C-2 of compounds **1** – **5** was deduced based on comparison of the vicinal coupling constants $J_{(1-2B)}$ and $J_{(2B-3)}$ which are very similar to the respective coupling constants of rocaglamide (**7**) (Nugroho *et al.*, 1997). Thus, compounds **1** – **5** should have the same absolute configuration at C-2 as rocaglamide (**7**).

All rocaglamide derivatives isolated from flowers of *A. odorata* are highly insecticidal to neonate larvae of *S. littoralis* when incorporated into artificial diet and offered to the larvae in a chronic feeding bioassay. The LC_{50}s, as well as EC_{50}s, of compounds **3** – **6** calculated from the respective dose response curves by probit analysis are given in Table II. The corresponding data of compounds **1** and **2,** previously isolated from *A. duperreana*, have already been reported (Nugroho *et al.*, 1997) and are included in Table II for comparison. The LC_{50} and EC_{50} values of the well known natural insecticide azadirachtin present in *Azadirachta indica* (Meliaceae) which was used as a positive control are also shown in Table II. From the rocaglamide derivatives isolated in this study compounds **1** and **4** are the most active ones exhibiting LC_{50}s of 1.5 and 1.6 ppm (2.9 and 3.2 nM) and identical EC_{50}s of 0.2 ppm (0.4 nM) respectively (Table II). Compounds **1** and **4** are thus only slightly less active than azadirachtin which shows an LC_{50} of 0.9 ppm (1.3 nM) and an EC_{50} of 0.04 ppm (0.06 nM). Based on the experiments of this, as well as on those of a previous study (Nugroho *et al.*, 1997), preliminary structure-activity relations can be drawn. The presence of an O-acetyl substituent as in compounds **2, 3** and **5,** decreases the insecticidal activity of rocaglamide derivatives compared to related compounds bearing a OH substituent at C-1 (e.g. **1** and **4**). Substitution at C-2 on the other hand appears to be less important since the rocaglaol derivative **6** is still considerably active (LC_{50}s 6.7 ppm [14.4 nM]) in spite of the lack of an aminoacyl substituent at C-2.

Table II. LC_{50} and EC_{50} values of insecticidal rocaglamide derivatives **1–6** and of azadirachtin towards neonate larvae of *Spodoptera littoralis*.

Compound	LC_{50} ppm (nM)	EC_{50} ppm (nM)
1	1.5 (2.9)	0.21 (0.40)
2	8.0 (14.2)	0.52 (0.92)
3	17.8 (32.4)	1.20 (2.19)
4	1.6 (3.2)	0.21 (0.43)
5	53.4 (97.1)	2.60 (4.73)
6	6.7 (14.4)	0.41 (0.88)
Azadirachtin	0.9 (1.3)	0.04 (0.06)

Chronic feeding experiments : Neonate larvae of *S. littoralis* (n = 20) were released on diet spiked with various concentrations of the analysed compounds (0.01–100 ppm). After six days of exposure, survival and weight of the surviving larvae were measured and compared to controls that had been exposed to diet treated with solvent (Me_2CO) only. From the dose-response curves LC_{50} and EC_{50} values were calculated by probit analysis.

Further studies on insecticidal rocaglamide derivatives are underway.

Acknowledgements

Financial support by grants of the DFG (SFB 251) and by the "Fonds der Chemischen Industrie" is gratefully acknowledged. We thank Prof. G. Bringmann (Univ. Würzburg) for helpful discussions.

Rembold H. (1989), in: Economic and Medicinal Plant Research (Wagner H., Hikino H. and Farnsworth N. R., eds.), Vol. **3**. Academic Press, London, pp. 57–72.

Klocke J. A. (1989), in: Economic and Medicinal Plant Research (Wagner H., Hikino H. and Farnsworth N. R., eds.), Vol. **3**. Academic Press, London, pp. 103–144.

Janprasert J., Satasook C., Sukumalanand P., Champagne D. E., Isman M. B. and Towers G. H. N. (1993), Rocaglamide, a natural benzofuran insecticide from *Aglaia odorata*. Phytochemistry **32**, 67.–69.

Ishibashi F., Satasook C., Isman M. B. and Towers G. H. N. (1993), Insecticidal 1*H*-cyclopentatetrahydro(b)benzofurans from *Aglaia odorata*. Phytochemistry **32**, 307–310.

Nugroho B. W., Edrada R. A., Güssregen B., Wray V., Witte L. and Proksch P. (1997), New insecticidal rocaglamide derivatives from *Aglaia duperreana* (Meliaceae). Phytochemistry **44**, 1455–1461.

Trost B. M., Greenspan P. D., Yang B. V. and Saulnier M. G. (1990), An unusual oxidative cyclization: a synthesis and absolute stereochemical assignment of (-) rocaglamide. J. Am. Chem. Soc. **112**, 9022–9024.

Kokpol U., Venaskulchai B., Simpson J. and Weavers R. T. (1994), Isolation and x-ray structure determination of a novel pyrimidinone from *Aglaia odorata*. J. Chem. Soc. Chem. Commun. 773–774.

Dumontet V., Thoison O., Omobuwajo O. R., Martin M.-T., Perromat G., Chiaroni A., Riche C., Pais M. and Sevenet T. (1996), New nitrogenous and aromatic derivatives from *Aglaia argentea* and *A. forbesii*. Tetrahedron **52**, 6931–6942.

Srivastava R. P. and Proksch P. (1991), Contact toxicity and feeding inhibitory activity of chromenes from Asteraceae against *Spodoptera littoralis* (Lepidoptera: Noctuidae). Entomol. Gener. **15** (4), 265–274.