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An insecticidal rocaglamide derivatives and related compounds from *Aglaia odorata* (Meliaceae)

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Abstract

Organic extracts of the twigs and leaves of *Aglaia odorata* yielded eight insecticidal cyclopentatetrahydrobenzofuran rocaglamide derivatives including three congeners which proved to be new natural products. Moreover, four new cyclopentatetrahydrobenzopyran aglain derivatives, as well as the known aminopyrrolidine odorine and odorinol, syringaresinol and flavonoid derivatives were also isolated. Structure elucidation of the new compounds is described and a rationale of the biosynthesis of the rocaglamide and aglain congeners is considered. The isolated rocaglamide derivatives exhibited strong insecticidal activity towards neonate larvae of the polyphagous pest insect *Spodoptera littoralis* when incorporated into artificial diet with LC_{50} values varying from 1.0–8.0 ppm. The most active compounds showed LC_{50} values between 1.0 and 1.1 ppm, comparable to those of the insecticide azadirachtin, which was used as a positive control. The remaining compounds isolated from *A. odorata* were inactive with regard to insecticidal activity.

Keywords: *Aglaia odorata*; Meliaceae; Benzofurans; Benzopyrans; Rocaglamide derivatives; Aglain derivatives; Structure elucidation; Natural insecticides; *Spodoptera littoralis*

1. Introduction

From several species of the genus *Aglaia* (Meliaceae), a series of rocaglamide derivatives which feature the cyclopentatetrahydrobenzofuran skeleton have been isolated and were shown to exhibit insecticidal activity (Janprasert et al., 1993; Ishibashi, Satasook, Isman, & Towers, 1993; Nugroho et al., 1997a, 1997b; Güssregen et al., 1997; Nugroho, 1997). This activity reported for the rocaglamide congeners is comparable to that of azadirachtin, a powerful natural insecticide that is of considerable commercial interest and has prompted us to screen for further active derivatives. To date, more than 24 rocaglamide derivatives have been isolated from *Aglaia* species (King et al., 1982; Janprasert et al., 1993; Ishibashi et al., 1993; Kokpol, Venaskulchai, Simpson, & Weavers, 1994; Dumontet et al., 1996; Ohse, Ohba, Yamamoto, Koyano, & Umezawa, 1996; Cui et al., 1997; Güssregen et al., 1997; Nugroho, 1997; Nugroho et al., 1997a; Nugroho et al., 1997b). Moreover, three aglain compounds (aglain A, B, and C), which possess a novel cyclopentatetrahydrobenzopyran skeleton, have

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been isolated from the bark of *A. forbesii* and from the leaves of *A. argentea* (Dumontet et al., 1996). In the present study, we have analyzed twigs and leaves of *Aglaia odorata* Lour. from Indonesia and report on the isolation of three new insecticidal rocaglamide compounds, as well as biosynthetically related insecticidally inactive compounds which include: four new aglain derivatives, aminopyrrolidine odorine and odorinol, syringaresinol and flavonoid derivatives.

2. Results and discussion

Crude methanolic extracts from the twigs and leaves of *A. odorata* exhibited significant insecticidal activity towards neonate larvae of the polyphagous pest insect *Spodoptera littoralis*. In a chronic feeding bioassay, at an arbitrarily chosen concentration of 1000 ppm of each extract incorporated into artificial diet, none of the insects were found to survive beyond the first 2 or 3 d of the experiment (data not shown). Consequently, both extracts were chosen for a bioassay-guided isolation of the active principles.

Repeated chromatographic separation of the twig extract yielded six insecticidal rocaglamide compounds (**1**–**6**), while from the leaf extract, four rocaglamide congeners (**1**, **4**, **7**, **8**), four aglain derivatives (**9**–**12**), the aminopyrrolidines odorine (**13**) and odorinol (**14**), syringaresinol (**15**) and flavonoid derivatives (**16**–**18**) were isolated. Based on their spectral characteristics and on comparison with published data, compounds **1**–**3** were readily identified as C-3′-hydroxyrocaglamide, C-1-*O*-acetyl-3′-hydroxyrocaglamide and C-3′-methoxyrocaglamide respectively, which have been previously isolated from the twigs of *A. duperreana* (Nugroho et al., 1997a). C-3′-methoxylrocaglaol (**4**) and C-3′-hydroxydidemethylrocaglamide (**7**) have been reported from the flowers of *A. odorata* (Güssregen et al., 1997) whereas compounds **5**, **6**, **8** and **9**–**12** are new natural products. For the structural elucidation of the rocaglamide derivatives (**1**–**8**) careful comparison of the ^{1}H and ^{13}C data with our previously published data (Güssregen et al., 1997; Nugroho et al., 1997a; Nugroho et al., 1997b) allowed substituent chemical shifts to be readily interpreted. In the case of the aglains our initial assumption was that these were rocaglamide derivatives and only involved differences in the amide substituent at C-2. However, this was clearly not the case. Although similar substituents were present to those of the rocaglamide systems considerable changes of chemical shifts were observed for signals associated with the benzofuran ring system (in particular the shifts of C-4a, C-8a and C-8b) that would not occur if the only substituent change was at C-2. Consequently complete sets of 2D spectra including HMQC and HMBC were recorded and these were used in conjunction with the literature data (Dumontet et al., 1996) to formulate the structures of the new compounds.

The MS and NMR spectral data of compounds **5**, **6** and **8** are comparable to those of the known rocaglamide derivatives which feature the cyclopentatetrahydrobenzofuran skeleton as the basic structure and differ only with regard to their substituents at C-1 and/or C-2 and/or C-3′. Compounds **5** and **6** prove to be the new C-3′-hydroxy derivatives of demethylrocaglamide and methylrocaglate, respectively, as shown by molecular ion peaks in EI-MS at *m*/*z* 507 and 508, which are 16 mu higher than those of demethylrocaglamide $[M]^{+}$ 491 and methylrocaglate $[M]^{+}$ 492. Inspection of the ^{1}H and ^{13}C NMR spectra allowed assignment of the hydroxyl substituent at C-3′ (Table 1). In the ^{1}H NMR spectrum, the presence of the hydroxyl substituent at C-3′ shifted the protons at C-2′ and C-6′ to higher field by 0.35 and 0.41 ppm, respectively. Investigation of the ^{1}H NMR spectra of several rocaglamide derivatives, showed empirically that hydroxylation at C-3′ causes a deshielding effect on the aromatic protons at ring B in the following order: H-2′ > H-6′ > H-5′.

Compound **8** was identified as a C-1-oxime, C-3′-methoxy derivative of methylrocaglate. In the ^{13}C NMR spectrum of **8**, the presence of an oxime substituent at C-1 caused, as expected, a large downfield shift (δ 153.0) compared to the C-1 resonance for methylrocaglate at δ 80.6. This substitution is also characterized by deshielding effects on C-2 and C-8b of ca. 6 and 23 ppm. However, no obvious change in the shift was observed for C-3. In its ^{1}H NMR spectrum, there is a loss of the H-1 resonance at ca. 4.90 ppm and H-2 was observed as a doublet at 3.90 ppm through coupling with H-3 at 4.32 ppm instead of the double doublet found in methylrocaglate. The presence of an additional methoxyl substituent at C-3′ (δ 3.64) caused a shielding effect on C-2′ and C-6′ of 0.34 and 0.20 ppm, respectively, while C-5′ was deshielded by 0.11 ppm. In contrast to the presence of a hydroxyl substituent at C-3′, methoxylation at C-3′ causes a deshielding of the aromatic protons of ring B in the following order: H-6′ > H-5′ > H-2′.

Compounds **9**–**12** are comparable with the aglain congeners described previously (Dumontet et al., 1996) which possess a cyclopentatetrahydrobenzopyran skeleton. The aglain derivatives differ from those of the rocaglamide congeners with regard to the structure of the two central ring systems. In contrast to the previously described aglain derivatives, the isolated congeners were substituted at C-19 and C-3′ in ring B. In addition to the considerable chemical shift differences in the benzofuran ring system compared to the rocaglamides noted above each of the ^{1}H spectra showed a singlet and two doublets (with couplings of 9.1 Hz for

Table 1
^{1}H and ^{13}C NMR data of rocaglamide derivatives **5**, **6** and **8**

	5	**6**	**8**
H-Atom			
1	4.79 (d, 6.0)	4.89 (d, 6.2)	
2	3.86 (dd, 5.5, 14.0)	4.01 (dd, 6.2, 14.2)	3.78 (d, 13.2)
3	4.31 (d, 14.3)	4.27 (d, 14.2)	3.50 (d, 13.2)
5	6.31 (d, 1.9)	6.32 (d, 1.9)	6.35 (d, 2.0)
7	6.20 (d, 1.9)	6.21 (d, 2.0)	6.25 (d, 2.0)
2′	6.79 (d, 2.0)	6.76 (d, 2.0)	6.77 (d, 3.4)
5′	6.66 (d, 8.5)	6.67 (d, 8.6)	6.79 (d, 9.9)
6′	6.73 (dd, 2.0, 8.5)	6.70 (dd, 2.1, 8.5)	6.91 (dd, 2.0, 8.4)
2″, 6″	7.01 (m)	6.95 (m)	6.95 (m)
3″, 5″	7.06 (m)	7.05 (m)	7.12 (m)
4″	7.06 (m)	7.05 (m)	7.12 (m)
OCH_3-6	3.87 (s)	3.86 (s)	3.87 (s)
OCH_3-8	3.89 (s)	3.87 (s)	3.91 (s)
OCH_3-3′			3.64 (s)
OCH_3-4′	3.76 (s)	3.76 (s)	3.78 (s)
CO–OCH_3		3.66 (s)	3.69 (s)
N–CH_3	2.69 (s)		
C-Atom			
1	80.7	80.7	153.0
2	52.8	52.2	57.1
3	56.8	56.4	57.2
3a	102.6	102.8	105.7
5	90.0	90.0	89.9
7	93.0	93.1	93.8
8a	109.4	109.3	110.0
8b	95.1	95.1	117.0
1′	130.2	130.1	128.7
2′	116.8	116.7	113.2
3′	146.0	146.0	149.3
5′	111.1	111.2	111.5
6′	120.8	120.7	121.4
1″	139.1	139.2	136.7
2″, 6″	128.5	129.1	129.4
3″, 5″	129.3	128.5	128.7
4″	127.2	127.2	128.0
4′, 8, 4a, 6	147.7	147.8	149.5
	159.4	159.3	160.3
	162.3	162.2	161.3
	165.2	165.3	165.3
Ar–OCH_3	56.2, 56.1, 56.0	56.2, 56.1, 56.0	56.1, 56.1, 56.3, 56.3
CO–OCH_3		52.5	57.5
C=O	173.4	172.6	171.7
N–CH_3	26.3		

9–**11** and 6.7 Hz for **12**) in the region usually associated with H-1 to H-3 of the rocaglamides. More importantly, in the long-range ^{13}C–^{1}H (HMBC) correlation there is a strong correlation of H-4 (H-2 of the rocaglamides) with C-5a (C-8a of the rocaglamides) that is not possible for the rocaglamide structure. The same spectrum allowed unambiguous identification of H-3/H-4 through correlation with the phenyl system and carbonyl system of the odorine/ol system (Table 2).

Based on these data for the isolated compounds and by comparison with ^{1}H and ^{13}C data recorded under the same conditions for aglain C isolated by Dumontet et al. (1996), compounds **9**, **10**, and **11** were readily identified as C-3′-hydroxyaglain C, C-19,C-3′-dihydroxyaglain C, and C-19-hydroxy, C-3′-methoxyaglain C, respectively. The ^{1}H and ^{13}C NMR spectra of **9**, **10**, and **11** are comparable but not identical to those of aglain C. The molecular ion peak [M]+ of **9** at m/z 646 as determined by EI-MS was 16 mass units higher and an additional aromatic hydroxyl substituent at C-3′ is evident from the changes in the ^{1}H and ^{13}C shifts of the B ring system, which were unambiguously assigned from the HMQC and HMBC spectra. Similar arguments identified the presence (ESI- or EI-MS) and position (NMR data) of the additional hydroxyl sub-

Table 2
^{1}H and ^{13}C NMR data of aglain derivatives **9**–**12**

	9	**10**	**11**	**12**
H-Atom				
3	4.41 (d, 9.1)	4.43 (d, 9.1)	4.48 (d, 9.1)	4.50 (d, 6.7)
4	4.23 (d, 9.1)	4.33 (d, 9.2)	4.39 (d, 9.3)	4.27 (d, 6.6)
7	6.17 (d, 2.3)	6.17 (d, 2.3)	6.18 (d, 2.3)	6.24 (d, 2.3)
9	6.08 (d, 2.2)	6.09 (d, 2.3)	6.11 (d, 2.2)	6.20 (d, 2.2)
10	4.59 (s)	4.57 (s)	4.58 (s)	4.20 (s)
13	6.71 (d, 5.5)	6.82 (d, 5.7)	6.82 (m)	5.05 (d, 4.8)
14A	1.91 (m)	1.91 (m)	1.92 (m)	1.25 (m)
14B	2.16 (m)	2.19 (m)	2.20 (m)	1.43 (m)
15A	1.89 (m)	1.92 (m)	1.93 (m)	1.79 (m)
15B	1.99 (m)	2.02 (m)	2.02 (m)	1.79 (m)
16A	3.25 (m)	3.28 (m)	3.28 (m)	3.28 (m)
16B	3.51 (m)	3.51 (m)	3.54 (m)	3.44 (m)
19	1.94 (m)			1.82 (m)
20A	1.35 (m)	1.49 (m)	1.52 (m)	1.17 (m)
20B	1.52 (m)	1.77 (m)	1.78 (m)	1.36 (m)
21	0.84 (t)	0.84 (t)	0.85 (t)	0.70 (t)
22	1.04 (d, 6.8)	1.22 (s)	1.24 (s)	0.84 (d, 6.9)
2′	7.05 (m)	7.05 (m)	7.20 (m)	7.60 (d, 2.1)
6′	7.05 (m)	7.05 (m)	7.20 (m)	7.74 (dd, 2.2, 8.6)
5′	6.64 (d, 8.3)	6.64 (d, 9.1)	6.75 (d, 8.6)	7.07 (d, 8.7)
2″, 6″	7.21 ('d', 7.3)	7.20 ('d', 6.9)	7.28 (m)	7.53 ('d', 7.2)
3″, 4″, 5″	7.01 (m)	6.96 (m)	7.00 (m)	7.30 (m)
OCH_3-6	3.84 (s)	3.85 (s)	3.85 (s)	3.94 (s)
OCH_3-8	3.78 (s)	3.79 (s)	3.80 (s)	3.90 (s)
OCH_3-4′	3.76 (s)	3.76 (s)	3.76 (s)	3.82 (s)
OCH_3-5′			3.54 (s)	
C-Atom				
1a	155.1	155.0	155.0	155.0
2	89.9	89.9	87.9	89.8
3	58.6	58.7	61.8	58.5
4	63.3	63.6	62.2	62.6
5	83.2	83.1	81.4	83.2
5a	107.0	106.9	113.8	107.0
6	159.8	159.8	157.5	159.9
7	92.7	92.7	93.1	92.8
8	162.7	162.7	161.8	162.7
9	95.1	95.0	95.2	95.0
10	81.4	81.3	83.6	81.3
11	172.2	172.1	171.4	172.1
13	64.9	65.0	64.9	64.9
14	35.1	35.2	34.3	35.1
15	21.9	21.8	21.4	21.9
16	47.2	47.3	46.9	47.3
18	177.7	178.2	178.1	177.7
19	76.2	42.8	42.8	76.1
20	32.4	28.6	27.4	34.3
21	8.3	12.0	12.0	8.2
22	26.6	17.6	17.0	26.7
1′	132.9	132.9	132.9	132.4
2′	117.7	117.7	115.6	115.3
3′	146.0	146.0	146.9	148.8
4′	147.9	147.9	148.8	149.3
5′	111.1	111.0	112.4	111.1
6′	122.0	122.0	120.3	122.7
1″	143.0	143.3	142.0	143.0
2″, 6″	131.5	131.6	131.8	131.6
3″, 5″	128.8	128.7	129.0	128.9
4″	126.9	126.9	127.3	122.8
OCH_3-6	56.5	56.5	56.7	56.5

Table 2 (*continued*)

	9	10	11	12
OCH_3-8	56.1	56.1	56.5	56.1
OCH_3-3′				56.2
OCH_3-4′	55.8	55.8	55.8	55.8

Compounds **9**, **10** and **12** show long-range correlations for the central cyclopentatetrahydrobenzopyran and nitrogen-containing systems that are characteristic and are as follows: C-2: H-3, H-10, H-2′, H-6′; C-3: H-4, H-10, H-2″/6″; C-4: H-3, H-10; C-5: H-4; C-5a: H-4, H-7, H-9, H-10; C-1a: H-9; C-11: H-3, H-4, H-13; C-13: H-14 A/B, H-15 A/B; C-14: H-13, H-15 A/B, H-16B; C-15: H-13, H-14, H-16 A/B; C-16: H-13, H-15B; C-18: H-13, H-20 A/B, H-22; C-19: H-20 A/B, H-21, H-22; C-20: H-21, H-22; C-21: H-20 A/B; C-22: H-20 A/B. The same spectra show the expected correlations for rings A, B and C that are also present in the rocaglamide derivatives.

stituent in **10** and the methoxyl substituent in **11**. Dumontet et al. (1996) have shown that the relative configuration at C-3 and C-4 can be established from the magnitude of the vicinal coupling constant between the respective protons. The value of 9.1 Hz found here for **9**–**11** is only compatible with the 3α, 4β-configuration. Similarly the shift of C-10 (δ ca. 81 ppm) is compatible with the aglain C configuration at this carbon (δ ca. 84 ppm) rather than the reverse configuration found in aglain A (δ ca. 74 ppm) (Dumontet et al., 1996).

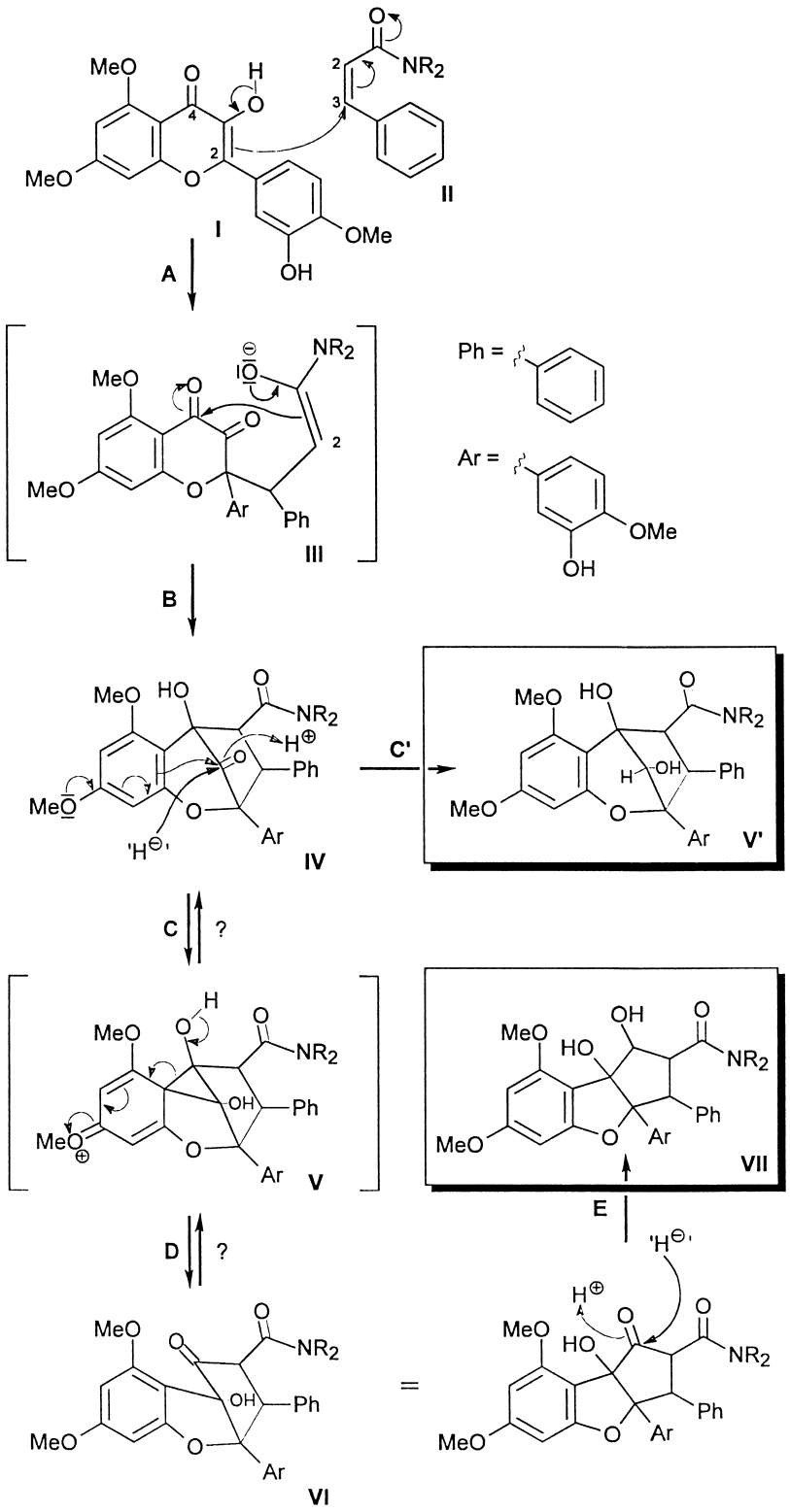
The same arguments indicated that compound **12** is the 3β,4α-stereoisomer of **9** and can be regarded as the equivalent aglain B derivative. The ^{1}H and ^{13}C NMR data show similar differences to those found between aglains C and B for those atoms that would be expected to be affected by the configurational change. Thus on going from **9** to **12** there is a pronounced high-field shift of H-10 (−0.73 ppm, lit. −0.65 ppm (Dumontet et al., 1996)), a decrease in the ^{3}J (3–4) to 6.7 Hz (lit. 5 Hz (Dumontet et al., 1996)), a low-field shift of C-5a (+6.9 ppm, lit. +6.3 ppm (Dumontet et al., 1996)), and a low-field shift of C-10 (+2.3 ppm, lit. +3.0 ppm (Dumontet et al., 1996)), which are similar to those reported in the literature for the change of aglain C to aglain B. There is a remarkable upfield shift for H-13 (assigned correctly from the HMBC spectrum) and also significant ^{1}H shifts in the other parts of the odorine moiety on going from **9** to **12**. These can only arise from changes in ring current effects, as no significant abnormalities are observed in the corresponding ^{13}C shifts. It can not be excluded, however, that the configuration at the odorine moiety has not changed although naturally occurring odorine is (+)-(*E*, 2*S*, 2′*R*)-2-methyl-*N*-[1′-(1″-oxo-3″-phenyl-prop-2″-enyl)pyrrolidin-2′-yl]butanamide (Babidge et al., 1980); in contrast both (+)- and (−)-odorinol have been detected in *A. odorata* (Janprasert et al., 1993; Hayashi, Lee, Hall, McPhail, & Huang, 1982).

It is tempting to assume that rocaglamides (**1**–**8**) and aglain derivates (**9**–**12**) arise biosynthethically from the simpler, but structurally related 'partial structures', cinnamic acid derivatives (**13** and **14**) and flavonoids (**16**–**18**). Our biosynthetic rationale is depicted in Scheme 1. According to our hypothesis, the initial *C,C*-connecting step (step A) between C-2 of the flavonoids of **I** (here **17**) and C-3 of the cinnamic amide **II** (e.g. **13** or **14**) is a Michael-type 1,4-addition of the enolate subunit of **I** to the α,β-unsaturated amide **II**. The C-2 atom of the resulting amide enolate of **III** can now attack C-4 of the previous flavonoid, which has now become a strongly activated carbonyl group, to close a 5-membered ring, giving rise to **IV** (step B). According to our concept, **IV** constitutes the biosynthetic key intermediate and precursor both to aglain and rocaglamide derivatives. **IV** can already be considered as a dehydroaglain derivative, and a simple reduction step (e.g. with 'H^-' possibly being NADPH or a related *H*-nucleophile), to give the corresponding aglain derivative **V**′ (step C′).

This reduction to give **V** stabilizes the strained molecule **IV**, which, as the key intermediate, may otherwise undergo a rearrangement by an intramolecular migration of the electron-rich substituted (phloroglucinol-type) aromatic ring from the previous C-4 to C-3 of the flavonoid. Mechanistically, this can be considered as an electrophyllic aromatic *ipso*-substitution via the cyclopropyl derivative **V** as the σ-complex (steps C and D), thus ultimately transforming the hydroxyketone **IV** into the isomeric hydroxyketone **VI**, which is already a dehydrorocaglamide derivative. Again, this possibly reversible process becomes definite by a stabilizing final reduction step (step E), to give rise to rocaglamide derivatives **VII**.

From these considerations, the search for the probably unstable, possibly interconverting intermediates **IV** and **VI** of our postulated biosynthesis of dehydroaglain and rocaglamide derivatives, seems rewarding.

All rocaglamide derivatives isolated from the twigs and leaves of *A. odorata* were assayed for insecticidal activity against the neonate larvae of *S. littoralis* by incorporating the compounds into an artificial diet over a range of different concentrations. Azadirachtin was used as a positive control. For these experiments,



Scheme 1. Proposed joint biosynthetic origin of aglain derivatives **V′** (such as **9**–**12**) and rocaglamide derivatives **VII** (such as **1**–**8**).

the LC_{50} and EC_{50} of each compound was calculated by probit analysis (Table 3). C-3′-methoxyrocaglamide (**3**) and C-3′-hydroxy derivatives of demethylrocaglamide and methylrocaglate (**5** and **6**) were found to be the most active compounds with LC_{50} values of 1.0, 1.1, and 1.1 ppm, whereas their EC_{50} values were determined to be 0.09, 0.18 and 0.27 ppm, respectively. Their insecticidal activities are comparable to that of

Table 3
LC_{50} and EC_{50} values of insecticide rocaglamide derivatives **1**–**8** and of azadirachtin towards neonates larvae of *Spodoptera littoralis*

Compound	LC_{50} (ppm)	EC_{50} (ppm)
1	1.5±0.65	0.21±0.08
2	8.0±1.44	0.52±0.08
3	1.0±0.35	0.09±0.03
4	6.7±1.75	0.41±0.12
5	1.1±0.60	0.18±0.08
6	1.1±0.62	0.27±0.04
7	1.6±0.55	0.21±0.07
8	1.3±0.34	0.21±0.05
Azadirachtin	0.9±0.35	0.04±0.08

Chronic feeding experiments: Neonate larvae of *S. littoralis* ($n=20$) were released on diet spiked with various concentrations of the analyzed compounds (0.01–30 ppm). After 6 d 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.

azadirachtin (Table 3). Among the rocaglamide derivatives tested, compounds **2** and **4** had the lowest insecticidal activity with LC_{50} values of 8.0 and 6.7 ppm, respectively (Table 3). This drop in activity can be attributed to the presence of an acetic acid moiety esterified at C-1 or the absence of a substituent at C-2. A similar trend has been noted in a recent study of other rocaglamide derivatives isolated from *A. duperreana* (Nugroho et al., 1997a), *A. elliptica* (Nugroho et al., 1997b) and *A. odorata* (Güssregen et al., 1997). All of the isolated aglain derivatives, the aminopyrrolidine odorine and odorinol, syringaresinol, and the flavonoid derivatives were found to be inactive at the range of concentrations analyzed. It is important to note that the benzopyran skeleton found in the aglain congeners resulted in the loss of insecticidal activity. This fact strongly suggests that the potency of the rocaglamide derivatives is due to the presence of the benzofuran structure that provides a suitable scaffold for the appropriate substituents. The experimental data presented here, as well as those of previous studies (Janprasert et al., 1993; Ishibashi et al., 1993; Güssregen et al., 1997; Nugroho, 1997; Nugroho et al., 1997a; Nugroho et al., 1997b), provide substantial evidence that rocaglamide derivatives are powerful natural insecticides and may have considerable potential as new lead structures for plant protection. Although the aglains proved negative in our tests their screening in other test systems would appear justified as one of their constituents, odorinol, has previously shown potential antitumor activity (Hayashi et al., 1982). Interest has also been shown in this direction for the rocaglamide related compounds (Ohse et al., 1996; Wu et al., 1997).

3. Experimental

3.1. Plant material

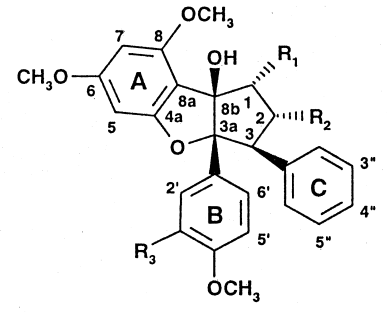
The plant material of *A. odorata* was supplied by the Bogor Botanical Garden in Indonesia. Voucher specimens are kept on file at the Julius-von-Sachs-Institute.

3.2. Extraction and isolation

Air dried twigs (2.0 kg dry wt.) and leaves of *A. odorata* (2.1 kg dry wt.) were ground and exhaustively extracted with MeOH. Following evaporation of the solvent, the extract was partitioned between MeOH/hexane, H_2O/CH_2Cl_2, H_2O/EtOAc and H_2O/water saturated *n*-butanol. Each fraction obtained was subjected to a bioassay with neonate larvae of *S. littoralis* (see below). From this bioassay the insecticidal activity was found to reside in the CH_2Cl_2- and EtOAc-fractions. Bioassay-guided fractionation of both fractions was achieved. Isolation of the compounds was accomplished by repeated chromatographic separation employing silica gel (Merck, Darmstadt, FRG) (mobile phase: CH_2Cl_2/iso-propanol 90:10 v/v, or hexan/$(CH_3)_2CO$ 1:1 v/v), Sephadex LH-20 (Sigma, Deisenhofen, FRG) (mobile phase: $(CH_3)_2$ CO) and Diol (Merck, Darmstadt, FRG) (mobile phase: hexan/EtOAc 3:7 v/v) as stationary phases. Final purification was obtained using RP-18 lobar columns (Merck, Darmstadt, FRG) (mobile phase: mixtures of MeOH and H_2O). Fractions were monitored by TLC on precoated silica gel plates (F254) (Merck, Darmstadt, FRG) (mobile phase: CH_2Cl_2/iso-propanol 90:10 v/v). Rocaglamide derivatives were detected by their absorbance under UV 254 nm or after spraying with the anisaldehyde reagent. Yields of the isolated compounds from the twig extracts (**1**–**6**) were: **1**: 14.5 mg, **2**: 7.0 mg, **3**: 6.0 mg, **4**: 38.8 mg, **5**: 5.0 mg, **6**: 13.0 mg. Yields of rocaglamide (**1**, **4**, **7** and **8**) and aglain (**9**–**12**) derivatives isolated from the leaf extracts were: **1**: 1.5 mg, **4**: 12.0 mg, **7**: 3.3 mg, **8**: 3.2 mg and **9**: 32.0 mg, **10**: 29.3 mg, **11**: 18.2 mg, **12**: 8.7 mg.

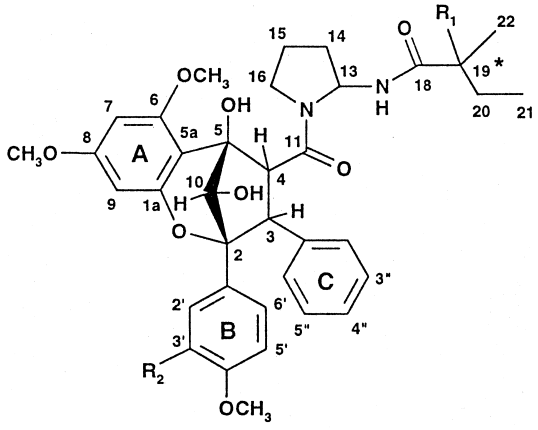
3.3. Spectroscopic identification of compounds

^1H and ^{13}C NMR spectra were recorded in CD_3OD on Bruker AM 300 or ARX 400 NMR spectrometers. EI-MS spectra (70 eV) were obtained by direct inlet on a Finnigan MAT 8430 instrument. FAB-MS spectra were recorded using glycerol as matrix. High resolution data were determined by peak matching at a resolution of approximately 10,000 (10% valley). The structures of **1**–**12** were determined from 1D (^1H and ^{13}C) and 2D [COSY, ^1H-detected direct (HMQC), and



Rocaglamide derivatives (1 - 8)

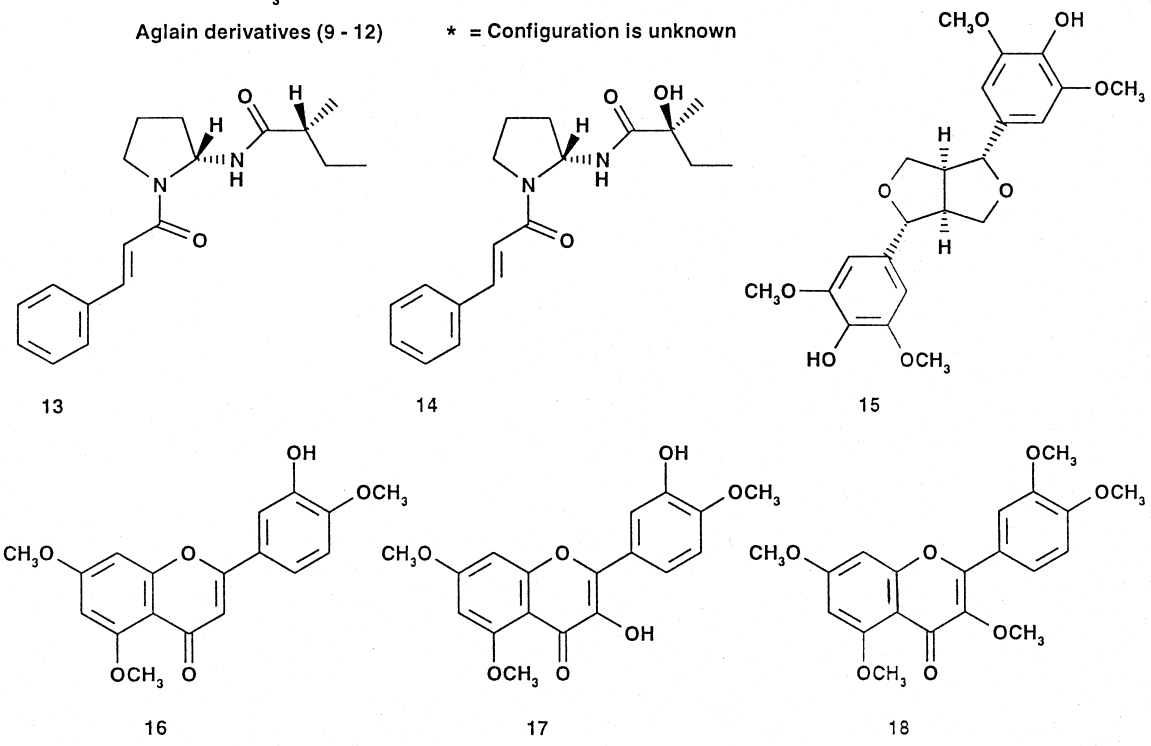
	R_1	R_2	R_3
1	OH	$CON(CH_3)_2$	OH
2	$OCOCH_3$	$CON(CH_3)_2$	OH
3	OH	$CON(CH_3)_2$	OCH_3
4	OH	H	OCH_3
5	OH	$CONHCH_3$	OH
6	OH	$COOCH_3$	OH
7	OH	$CONH_2$	OH
8	=NOH	$COOCH_3$	OCH_3



Aglain derivatives (9 - 12)

	R_1	R_2	
9	H	OH	[H-3α,H-4β,13S]
10	OH	OH	[H-3α,H-4β,13S]
11	OH	OCH_3	[H-3α,H-4β,13S]
12	H	OH	[H-3β,H-4α,13S]

* = Configuration is unknown



13 14 15

16 17 18

Fig. 1. Structures of rocaglamide (**1-8**) and aglain (**9-12**) derivatives, the aminopyrrolidines odorine (**13**) and odorinol (**14**), syringaresinol (**15**) and flavonoid derivatives (**16-18**) isolated from twigs and leaves of *A. odorata*.

long-range (HMBC) ^{13}C–^{1}H correlations] NMR data (Fig. 1).

3.4. Experiments with insects

Larvae of *Spodoptera littoralis* were from a laboratory colony reared on artificial diet under controlled conditions at 26°C as described previously (Srivastava, & Proksch, 1991). Feeding studies were conducted with neonate larvae ($n=20$ for each treatment). Neonate larvae were kept on diet treated with various concentrations of the analyzed compounds (0.01; 0.10; 0.25; 0.50; 0.75; 1.0; 2.0; 4.0; 6.0; 8.0; 10.0; 15.0; 20.0; 25.0; and 30.0 ppm). After 6 d of exposure to the sample-treated diet, mortality rate and weight of the surviving larvae were recorded and compared with the controls. Control diet was prepared with the carrier solvent (acetone). LC_{50}s and EC_{50}s were calculated from the dose–response curves by probit-analysis. Azadirachtin, which was used as a positive control, was commercially available from Roth (Karlsruhe, FRG).

3.5. Compound **1**

$[\alpha]_D^{20}-89.7°$ ($c=0.21$, $CHCl_3$). CD: 217 nm ($\Delta\varepsilon$ −13). EI-MS (*m*/*z*, rel. Int.): 521 [M]+ (24), 503 (22), 406 (55), 329 (70), 316 (69), 301 (27), 283 (20), 181 (50), 176 (100), 131 (16).

3.6. Compound **2**

$[\alpha]_D^{20}-83.3$ ($c=0.45$, $CHCl_3$). CD: 217 nm ($\Delta\varepsilon$ −12). EI-MS (*m*/*z*, rel. Int.): 563 [M]+ (53), 545 (37), 503 (95), 485 (50), 458 (68), 431 (48), 329 (76), 316 (100), 301 (50), 283 (79), 181 (86), 176 (39), 131 (17).

3.7. Compound **3**

$[\alpha]_D^{20}-64.6$ ($c=0.18$, $CHCl_3$). CD: 218 nm ($\Delta\varepsilon$ −17). EI-MS (*m*/*z*, rel. Int.): 535 [M]+ (10), 517 (8), 420 (40), 343 (48), 330 (60), 315 (23), 235 (27), 181 (41), 176 (100), 131 (19).

3.8. Compound **4**

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

3.9. Compound **5**

$[\alpha]_D^{20}-59.5°$ ($c=0.25$, $CHCl_3$). CD: 218 nm ($\Delta\varepsilon$ −12), 230 nm ($\Delta\varepsilon$ −4). EI-MS (*m*/*z*, rel. Int.): 507 [M]+ (20), 489 (22), 458 (10), 431 (14), 406 (48), 329 (65), 316 (100), 301 (36), 283 (28), 243(24), 181 (54), 162 (83).

3.10. Compound **6**

$[\alpha]_D^{20}-54.9°$ ($c=0.18$, $CHCl_3$). CD: 217 nm ($\Delta\varepsilon$ −15). EI-MS (*m*/*z*, rel. Int.): 508 [M]+ (15), 490 (6), 406 (10), 329 (24), 316 (100), 301 (30), 283 (34), 218 (10), 181 (24).

3.11. Compound **7**

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

3.12. Compound **8**

$[\alpha]_D^{20}-34.3°$ ($c=0.13$, $CHCl_3$). CD: 217 nm ($\Delta\varepsilon$−13), 234 nm ($\Delta\varepsilon$−6), 242 nm (sh) ($\Delta\varepsilon$−3). FAB-MS (Glycerol as Matrix): 536 [M–H]+. EI-MS (*m*/*z*, rel. Int.): 535 [M]+ (3), 419 (5), 343 (18), 330 (100), 315 (32), 287 (10), 181 (22), 165 (12).

3.13. Compound **9**

$[\alpha]_D^{20}-103.4°$ ($c=0.43$, $CHCl_3$). CD: 227 nm (sh) ($\Delta\varepsilon$−7), 238 nm (sh) ($\Delta\varepsilon$−8), 243 nm ($\Delta\varepsilon$−10). EI-MS (*m*/*z*, rel. Int.): 646 [M]+ (6), 640 (30), 545 (40), 476 (85), 458 (84), 431 (100), 343 (8), 330 (22), 329 (100), 328 (33), 200 (28), 181 (35), 131 (38), 103 (12).

3.14. Compound **10**

$[\alpha]_D^{20}-86.0°$ ($c=0.18$, $CHCl_3$). CD: 225 nm (sh) ($\Delta\varepsilon$−7), 235 nm (sh) ($\Delta\varepsilon$−12), 242 nm ($\Delta\varepsilon$−15). ESI-MS (*m*/*z*, rel. Int.): 663 [M+H]+ (48), 645 (8), 619 (8), 548 (8), 546 (23), 509 (8), 329 (100).

3.15. Compound **11**

$[\alpha]_D^{20}-111.1°$ ($c=0.18$, $CHCl_3$). CD: 222 nm (sh) ($\Delta\varepsilon$−5), 234 nm (sh) ($\Delta\varepsilon$−5), 242 nm ($\Delta\varepsilon$−6). EI-MS (*m*/*z*, rel. Int.): 676 [M]+ (5), 559 (30), 489 (18), 473 (42), 472 (58), 406 (42), 405 (100), 368 (12), 360 (18), 359 (25).

3.16. Compound **12**

$[\alpha]_D^{20}-11.4°$ ($c=0.25$, $CHCl_3$). CD: 222 nm ($\Delta\varepsilon$−9), 235 nm (sh) ($\Delta\varepsilon$−4), 242 nm (sh) ($\Delta\varepsilon$−4). EI-MS (*m*/*z*, rel. Int.): 646 [M]+ (0.2), 545 (1), 476 (3), 330 (22), 329 (100), 328 (70), 301 (22), 200 (50), 131 (38), 103 (3).

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