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New insecticidal rocaglamide derivatives from flowers of *Aglaia duperreana* (Meliaceae)

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Abstract

Flowers of *Aglaia duperreana* collected in Vietnam yielded thirteen insecticidal cyclopentatetrahydrobenzofuran derivatives of the rocaglamide type including five compounds which proved to be new natural products. Structure elucidation of the new compounds and their insecticidal activity against larvae of the pest insect *Spodoptera littoralis* are described. Most of the isolated rocaglamide derivatives exhibited strong to moderate insecticidal activity. The most active compounds were similar with regard to their bioactivity to the well known natural insecticide azadirachtin. However, replacement of the OH-group at C-8b (that is a characteristic structural feature of most known rocaglamide congeners) by an OC_2H_5-substituent as present in two of the isolated new derivatives was found to result in a loss of insecticidal activity.

Keywords: *Aglaia duperreana*; Meliaceae; Benzofurans; Rocaglamide derivatives; Structure elucidation; Natural insecticides; *Spodoptera littoralis*

1. Introduction

In our continuing search for new insecticidal compounds of plant origin we have recently reported on rocaglamide derivatives from *Aglaia* species collected in Indonesia and Vietnam (Güssregen et al., 1997; Nugroho et al., 1997a, 1997b, 1999). The genus *Aglaia* (Meliaceae) consists of some 130 species that are dioecious trees or shrubs and are widely distributed in the Indo-Malaysian region (Pannell, 1992; Ridley, 1922). Rocaglamide derivatives (e.g. **1**–**13**, Fig. 1) are unusual aromatic compounds featuring a cyclopentatetrahydrobenzofuran skeleton and are strictly confined to members of the genus *Aglaia*. Recently we provided a rationale for the biosynthesis of these unusual benzofurans featuring naturally occuring flavonoid and cinnamic acid amides as building blocks for the rocaglamide skeleton (Nugroho et al., 1999).

Rocaglamide type compounds are exceptionally strong natural insecticides with several of them being comparable to the well known natural insecticide azadirachtin isolated from the Neem tree (e.g. Nugroho et

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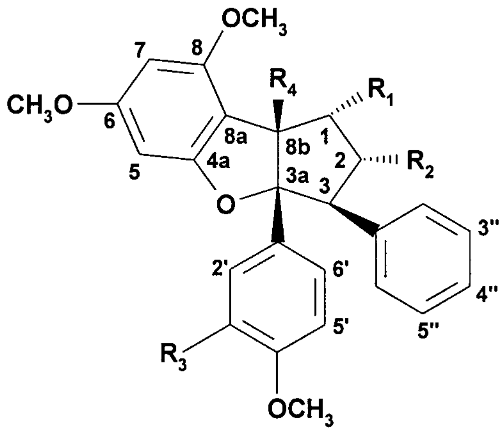
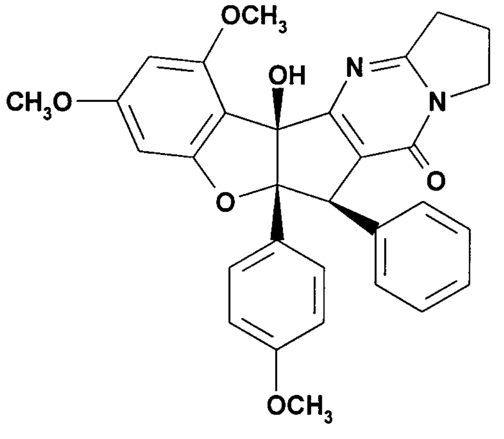


Rocaglamide derivatives (1 - 11)

	R_1	R_2	R_3	R_4
1	OH	$CON(CH_3)_2$	OH	OH
2	$OCOCH_3$	$CON(CH_3)_2$	OH	OH
3	OH	$CON(CH_3)_2$	OH	OC_2H_5
4	OH	$CONHCH_3$	H	OH
5	$OCOCH_3$	$CONHCH_3$	H	OH
6	$OCOCH_3$	$CONHCH_3$	OH	OH
7	OH	$CONHCH_3$	OH	OC_2H_5
8	$OCOCH_3$	$CONH_2$	H	OH
9	OH	$COOCH_3$	H	OH
10	$OCOCH_3$	$COOCH_3$	H	OH
11	$OCOCH_3$	$COOCH_3$	OH	OH



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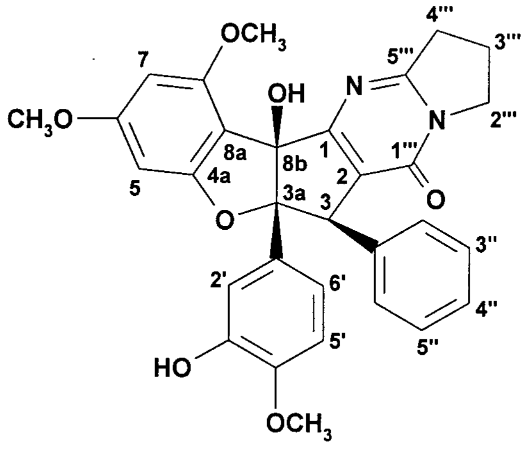

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Fig. 1. Structures of rocaglamide derivatives (**1**–**13**) isolated from flowers of *Aglaia duperreana*.

al., 1997a). The benzofuran skeleton appears to be an essential prerequisite for the insecticidal activity of rocaglamide derivatives since structurally closely related analogues, so called aglains, that differ from the former by the presence of a pyran vs. a furan ring are devoid of insecticidal activity (Nugroho et al., 1999). In the present study we have analysed flowers of *A. duperreana* Pierre collected in Vietnam and report on the isolation of 13 rocaglamide derivatives including five new compounds as well as on the insecticidal activity of the new rocaglamide derivatives against larvae of the pest insect *Spodoptera littoralis*.

Table 1
^{1}H NMR data of rocaglamide derivatives **3**, **7**, **8**, **10** and **13**

H-Atom	**3**	**7**	**8**	**10**	**13**
1	5.13 (d, 7.4)	4.93 (d, 7.4)	6.13 (d, 5.8)	6.13 (d, 5.3)	
2	4.10 (dd, 7.4, 13.7)	3.83 (dd, 7.0, 14.5)	4.06 (dd, 5.8, 14.4)	4.27 (dd, 5.3, 14.4)	
3	4.21 (d, 13.7)	4.11 (d, 14.5)	4.22 (d, 14.4)	4.18 (d, 14.5)	4.49 (s)
5	6.39 (d, 2.0)	6.36 (d, 2.0)	6.31 (d, 2.0)	6.32 (d, 2.0)	6.32 (d, 2.0)
7	6.27 (d, 2.0)	6.25 (d, 2.0)	6.16 (d, 1.9)	6.14 (d, 2.0)	6.17 (d, 2.0)
2′	6.73 (m)	6.71 (m)	7.42 (‘d’, 9.1)	7.16 (‘d’, 9.0)	6.69 (d, 2.1)
3′			6.66 (‘d’, 9.1)	6.12 (‘d’, 9.0)	
5′	6.73 (m)	6.71 (m)	6.66 (‘d’, 9.1)	6.12 (‘d’, 9.0)	6.56 (d, 8.5)
6′	6.73 (m)	6.71 (m)	7.42 (‘d’, 9.1)	7.16 (‘d’, 9.0)	6.63 (dd, 2.1, 8.4)
2″,6″	6.82 (m)	6.88 (m)	6.99 (m)	6.93 (m)	6.90 (m)
3″,4″,5″	7.03 (m)	7.05 (m)	7.05 (m)	7.08 (m)	7.05 (m)
OCH_3-6	3.89 (s)	3.89 (s)	3.78 (s)	3.86 (s)	3.84 (s)
OCH_3-8	3.92 (s)	3.91 (s)	3.86 (s)	3.87 (s)	3.85 (s)
OCH_3-3′					
OCH_3-4′	3.80 (s)	3.80 (s)	3.71 (s)	3.71 (s)	3.73 (s)
CO-OCH_3				3.66 (s)	
N–CH_3	2.87 (s); 3.34 (s)	2.64 (s)			
OCO–CH_3			1.89 (s)	1.89 (s)	
O–$\mathit{CH}_2$$CH_3$	2.68 (dq, 8.8, 7.0), 2.43 (dq, 8.8, 7.1)	2.71 (dq, 8.8, 7.0), 2.38 (dq, 8.9, 7.1)			
O–$CH_2$$\mathit{CH}_3$	0.71 (t, 7.0)	0.72 (t, 7.0)			
2‴A					4.18 (m)
2‴B					4.12 (m)
3‴A					2.40 (m)
3‴B					2.34 (m)
4‴A					3.36 (m)
4‴B					3.31 (m)

2. Results and discussion

Repeated chromatographic separation of a flower extract from *A. duperreana* resulted in the isolation of thirteen insecticidal rocaglamide derivatives (Fig. 1). Based on their spectral characteristics and on comparison with published data, compounds **1** and **2** were readily identified as C-3′-hydroxyrocaglamide and C-1-*O*-acetyl-3′-hydroxy-rocaglamide, respectively, which had previously been isolated from twigs of *A. duperreana* (Nugroho et al., 1997a). Compounds **4** and **9** were the known demethylrocaglamide and methylrocaglate both previously reported from the leaves of *A. odorata* (Ishibashi, Satasook, Isman & Towers, 1993). Compounds **6** and **11** were identified as C-1-*O*-acetyl-3′-hydroxydemethylrocaglamide and C-1-*O*-acetyl-3′-hydroxymethyl-rocaglate, respectively. Both compounds had previously been isolated from flowers of *A. odorata* (Güssregen et al., 1997). Compound **5** was the known C-1-*O*-acetyldemethylrocaglamide whereas compound **12** featured an unusual pyrimidinone unit. Both derivatives had previously been reported from the roots of *A. duperreana* (Hiort, 1998) or roots of *A. odorata* (Kokpol, Venaskulchai, Simpson & Weavers, 1994), respectively. Compounds **3**, **7**, **8**, **10** and **13** proved to be new natural products that differed from known rocaglamide derivatives with regard to their substituents at C-1, C-2, C-3′ and/or C-8b.

Compounds **3** and **7** turned out to be new and unusual rocaglamide congeners featuring an OC_2H_5-substituent at position C-8b instead of an OH-group as is usually encountered in this group of natural products (e.g. compounds **1**, **2**, **4**–**6** and **8**–**11**, Fig. 1). Presence of an OC_2H_5-substituent in compounds **3** and **7** was evident from inspection of their respective ^{1}H NMR spectra (Table 1) that featured characteristic signals at 0.7 ppm (–$OCH_2\mathit{CH}_3$–) and at circa 2.4 and 2.7 ppm (–$O\mathit{CH}_2CH_3$–). The facts that the CH_2 protons are not equivalent as well as their unusual shifts indicate that they are close to an asymmetric center and that they suffer shielding from ring current effects. These two prerequisites are only met when the OCH_2CH_3-group is positioned at C-8b. This assignment is further confirmed by comparing the spectral data for compounds **3** and **7** with those recently reported in the literature (Dumontet et al., 1996) for another rocaglamide derivative that also possessed an OCH_2CH_3-group at position C-8b.

Further support for the assignment of the OCH_2CH_3-group at position C-8b comes from inspection of the mass spectra of compounds **3** and **7** and of those obtained from structural analogues featuring an OH-group at C-8b. The mass spectra of **3** and **7** show

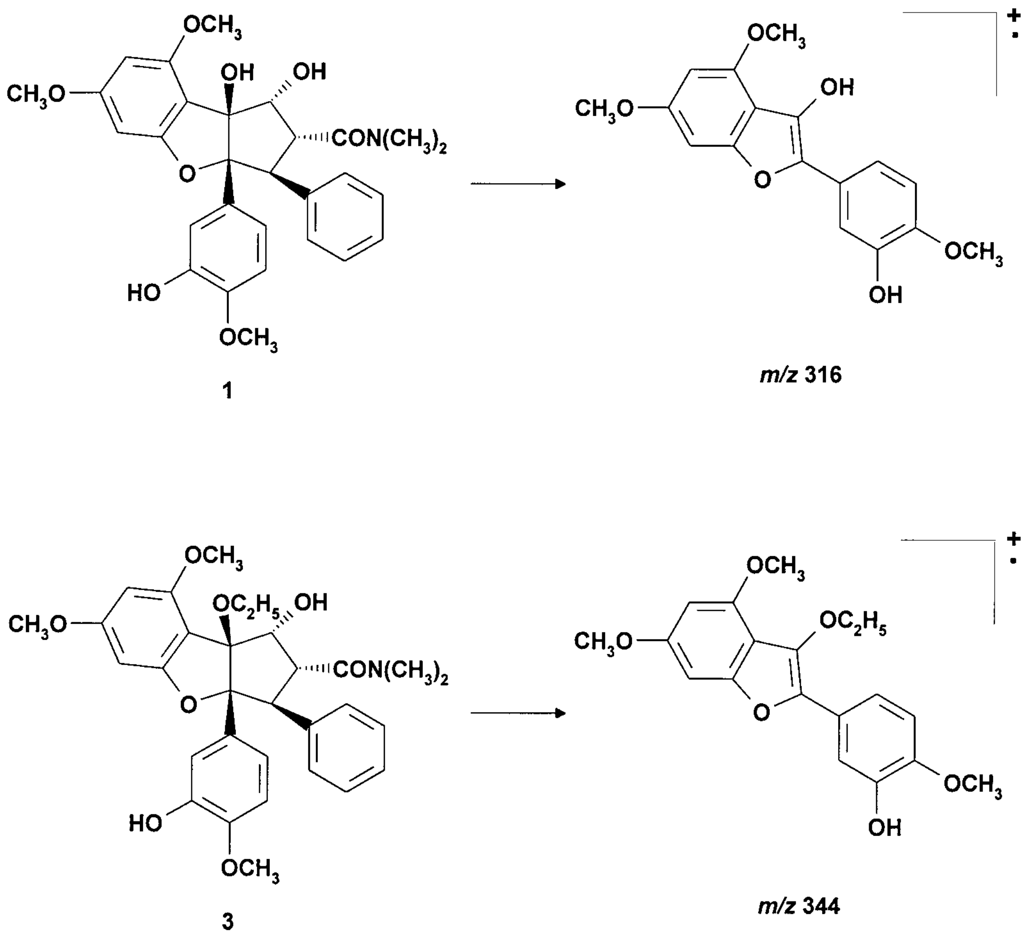

Fig. 2. Plausible structures of ions *m/z* 316 and 344 arising from fragmentation of compounds **1** and **3** under EI-mass spectrometry.

a characteristic fragment at *m/z* 344 which is indicative for the substitution patterns of rings A and B and of the furan ring as exemplified in Fig. 2 by comparing diagnostic fragments of compound **3** and of the structural analogue **1**.

An OCH_2CH_3-group as encountered in compounds **3** and **7** is a rare structural feature among natural products raising suspicion that **3** and **7** are in fact artefacts arising from **1** or **4** respectively. However, incubation of the suspected precursors **1** and **4** overnight either in EtOAc or in EtOH failed to give even traces of **3** or **7** thus strengthening the notion that the latter compounds are in fact true natural products.

Compounds **8** and **10** proved to be the new C-1-*O*-acetyl derivatives of didemethyl-rocaglamide and methylrocaglate, respectively. Presence of an acetate substituent in both compounds was evident from their respective mass and ^1H NMR spectra. The molecular ions of compounds **8** and **10** were observed at *m/z* 519 and *m/z* 534. The mass spectra of compounds **8** and **10** furthermore revealed characteristic fragments at *m/z* 459 and *m/z* 474, respectively, which are indicative for

Table 2
LC_{50} and EC_{50} values of rocaglamide derivatives **3**, **7**, **8**, **10** and **13** and of azadirachtin towards neonate larvae of *Spodoptera littoralis*[a]

Compound	LC_{50} (ppm)	EC_{50} (ppm)
1	1.50[c]	0.21[c]
3	N.A.[b]	N.A.[b]
4	1.30[c]	0.27[c]
7	N.A.[b]	N.A.[b]
8	1.97	0.14
10	6.62	1.03
13	5.70	0.31
azadirachtin	0.9	0.04

[a] Chronic feeding experiments: Neonate larvae of *S. littoralis* ($n = 20$) were released on diet spiked with various concentrations of the analyzed compounds (0.01-50 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.

[c] LC_{50} and EC_{50}-values of compounds **1** and **4** are from a previous study (Nugroho et al., 1997a).

[b] Not active at concentrations ≤ 50 ppm.

the loss of acetic acid. Assignment of the acetate unit at C-1 was evident from inspection of the ^{1}H NMR spectra of compounds **8** and **10** (Table 1). The resonances of H-1 of compounds **8** and **10** (6.13 ppm) had suffered large downfield shifts compared to the signal of H-1 of the corresponding parent compounds didemethylrocaglamide (4.72 ppm (Dumontet et al., 1996)) and methylrocaglate (5.02 ppm (Ishibashi et al., 1993)), both of which have an OH-subtituent at C-1.

Compound **13** which is characterized by a rare pyrimidinone unit proved to be the new C-3′-hydroxy derivative of the known congener **12** previously isolated from ground roots of *A. odorata* (Kokpol et al., 1994). Inspection of the ^{1}H and ^{13}C NMR spectra of **13** allowed assignment of the hydroxyl substituent at C-3′. In the ^{1}H NMR spectrum, the presence of the hydroxyl substituent at C-3′ shifted the resonances of protons at C-2′ and C-6′ to higher field by 0.39 ppm and 0.45 ppm respectively, compared to the known congener **12** (Kokpol et al., 1994).

The new rocaglamide derivatives isolated from flowers of *A. duperreana* were incorporated into artificial diet over a range of concentrations and assayed for insecticidal activity against neonate larvae of *S. littoralis*. The well known insecticidal compound azadirachtin was included in these experiments as a positive control. The LC_{50} and EC_{50} of each compound was calculated by probit analysis (Table 2). The LC_{50} and EC_{50}-values of the known rocaglamide (0.9 and 0.08 ppm) had already been reported before (Nugroho et al., 1997a). From the new compounds investigated C-1-*O*-acetyldidemethylrocaglamide (**8**) was the most active derivative with LC_{50} and EC_{50} values of 1.97 ppm and 0.14 ppm, respectively. Compound **8** is thus almost comparable to azadirachtin with regard to its insecticidal activity (Table 2). Rocaglamide derivatives **10** and **13** were less active in comparison (LC_{50} values 6.62 and 5.70 ppm, respectively) (Table 2). The most remarkable finding, however, was that compounds **3** and **7** were completely inactive in the range of concentrations analyzed (0.01–50 ppm) and thus represent the first known rocaglamide derivatives that are devoid of insecticidal activity. Since structurally closely related congeners that differ from **3** or **7** merely by the presence of an OH- rather than an OC_2H_5-substituent at C-8b show pronounced insecticidal activity against larvae of *S. littoralis* (Güssregen et al., 1997; Nugroho et al., 1997a; 1997b; 1999) the nature of the substituent at position C-8b of the furan ring seems to be an important structural feature influencing the insecticidal activity of the resulting rocaglamide derivatives. This observation is in congruence with the recent finding that aglain derivatives which differ from rocaglamide type compounds by a benzopyran rather than a benzofuran ring system are likewise devoid of insecticidal activity (Nugroho et al., 1999), thus pointing to the benzofuran nucleus as a key pharmacophore of rocaglamide compounds.

3. Experimental

3.1. Plant material

Flowers of *A. duperreana* were collected at a plantation in Ho Chi Minh City (Vietnam) in June of 1997. Voucher specimens are kept on file in the Julius-von-Sachs-Institute für Biowissenschaften, Universität Würzburg.

3.2. Extraction and isolation

Air dried flowers of *A. duperreana* (1 kg dry wt.) were ground and exhaustively extracted with MeOH. Following evaporation of the solvent the extract was partitioned between MeOH/hexane and H_2O/EtOAc (water saturated). 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 EtOAc-fraction. Bioassay-guided fractionation of the EtOAc-fraction was achieved through 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: $(CH_3)_2CO$) 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 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 of compounds were: **1**: 14.1 mg; **2**: 16.0 mg; **3**: 1.1 mg; **4**: 1.0 mg; **5**: 9.1 mg; **6**: 17.7 mg; **7**: 1.7 mg; **8**: 1.6 mg; **9**: 5.2 mg; **10**: 1.7 mg; **11**: 4.7 mg; **12**: 6.4 mg; **13**: 14.7 mg; respectively.

3.3. Spectroscopic identification of compounds

^{1}H 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. CD spectra were recorded in EtOH on Jobin Yvon Dichrograph CD 6.

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 analysed compounds (0.01; 0.05; 0.10; 0.25; 0.50; 0.75; 1.0; 1.5; 2.0; 2.5; 3.0; 5.0; 10.0; 15.0; 20.0 and 50.0 ppm). After 6 days survival and weight of the surviving larvae were recorded and compared with controls. Control diet was prepared with the carrier $(CH_3)_2CO$ only. LC_{50} and EC_{50} values were calculated from dose-response curves by probit-analysis.

Compound **3**: CD: 220 nm ($\Delta\varepsilon$-13). EI-MS (*m*/*z*, rel.Int.): 549 $[M]^+$ (20), 503 (18), 474 (10), 459 (10), 434 (10), 396 (15), 345 (28), 344 (42), 315 (32), 311 (16), 193 (10), 176 (100), 151 (14), 131 (27), 103 (10), 72 (20), 55 (14).

Compound **7**: CD: 219 nm ($\Delta\varepsilon$-5). EI-MS (*m*/*z*, rel.Int.): 535 $[M]^+$ (18), 489 (14), 459 (18), 346 (24), 345 (100), 344 (78), 315 (48), 193 (24), 162 (78), 151 (24), 131 (20), 83 (12), 60 (13), 55 (19).

Compound **8**: CD: 213 nm ($\Delta\varepsilon$-4). EI-MS (*m*/*z*, rel.Int.): 519 $[M]^+$ (8), 459 (56), 442 (32), 415 (30), 390 (18), 313 (82), 300 (100), 285 (50), 282 (27), 181 (37), 135 (24).

Compound **10**: CD: 216 nm ($\Delta\varepsilon$-8). EI-MS (*m*/*z*, rel.Int.): 534 $[M]^+$ (14), 474 (15), 456 (4), 442 (4), 415 (6), 390 (9), 320 (15), 313 (48), 300 (100), 284 (42), 283 (39), 282 (74), 192 (9), 181 (26), 135 (15), 131 (6).

^{13}C NMR (CD_3OD): δ 80.4 (d, C-1), 50.9 (d, C-2), 57.8 (d, C-3), 102.4 (s, C-3a), 161.9 (s, C-4a), 89.5 (d, C-5), 165.5 (s, C-6), 92.8 (s, C-7), 159.8 (s, C-8), 108.5 (s, C-8a), 94.5 (s, C-8b), 129.1 (s, C-1′), 130.2 (d, C-2′,6′), 113.3 (d, C-3′,5′), 160.0 (s, C-4′), 138.4 (s, C-1″), 129.0 (d, C-2″,6″), 128.7 (d, C-3″,5″), 127.5 (d, C-4″), 171.8 (s, CO–OCH_3), 170.9 (s, $O\mathit{CO}$-CH_3), 20.8 (q, OCO--CH_3), 52.5, 56.1, 55.7, 55.4 (q, 4 × OCH_3).

Compound **13**: CD: 216 nm ($\Delta\varepsilon$-4). EI-MS (*m*/*z*, rel.Int.): 540 $[M]^+$ (74), 522 (24), 509 (13), 463 (10), 389 (72), 386 (100), 359 (28), 343 (15), 315 (22), 181 (20), 151 (31), 125 (14), 95 (8), 83 (11), 44 (12).

^{13}C NMR (CD_3OD): δ 161.2 (s, C-1), 90.1 (s, C-2), 58.4 (d, C-3), 106.9 (s, C-3a), 162.0 (s, C-4a), 93.5 (d, C-5), 165.3 (s, C-6), 90.0 (d, C-7), 159.8 (s, C-8), 108.8 (s, C-8a), 122.0 (s, C-8b), 129.5 (s, C-1′), 116.3 (d, C-2′), 145.9 (s, C-3′), 148.0 (s, C-4′), 111.1 (d, C-5′), 120.5 (d, C-6′), 137.5 (s, C-1″), 130.5 (d, C-2″,6″), 128.2 (d, C-3″,5″), 127.4 (d, C-4″), 169.7 (s, C-1‴), 48.1 (t, C-2‴), 20.3 (t, C-3‴), 33.4 (t, C-4‴), 166.5 (s, C-5‴), 56.2, 56.4, 55.7 (q, 3 × OCH_3).

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