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INSECTICIDAL ROCAGLAMIDE DERIVATIVES FROM *AGLAIA DUPPEREANA*

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Key Word Index—*Aglaia duppereana*; Meliaceae; twigs; rocaglamide derivatives; structural elucidation; natural insecticides; *Spodoptera littoralis*.

Abstract—Twigs of *Aglaia duppereana* collected in Vietnam yielded the cyclopentatetrahydrobenzofuran, rocaglamide, and also six of its congeners. Whereas three of the isolated compounds were already known, four rocaglamide derivatives were new natural products. Elucidation of their structures and absolute configurations is described. All the rocaglamide derivatives isolated exhibited strong insecticidal activity towards neonate larvae of the polyphagous pest insect *Spodoptera littoralis* when incorporated into artificial diet. The LC_{50} for rocaglamide, which was one of the most active compounds encountered in this study, was 0.9 ppm, identical to that of azadirachtin used as a positive control in feeding experiments. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Aglaia* consists of some 130 species that are dioecious trees or shrubs with small fragrant flowers [1, 2]. Species of *Aglaia* form an important element of the moist tropical forest in the Indo-Malaysian region [3]. Several species, including *A. odorata*, are traditionally used in folk medicine in south-east Asia [4–6], for example, as a heart stimulant, febrifuge, for the treatment of coughs, inflammations and injuries.

More recently, extracts from *Aglaia* species were shown to exhibit significant insecticidal activity towards several pest species [7–9], suggesting that these plants may be new sources of natural insecticides. Aromatic compounds with a cyclopentatetrahydrobenzofuran skeleton, such as rocaglamide (**1**, Fig. 1) were subsequently shown to be responsible for the pronounced insecticidal activity of extracts derived from *A. odorata* [10, 11]. In the present study, we have extended the search for insecticidal rocaglamide derivatives to *A. duppereana* Pierre collected in Vietnam and report on the isolation of four new, and also three known rocaglamide derivatives, all exhibiting pronounced insecticidal activity towards neonate larvae of the polyphagous pest insect *Spodoptera littoralis* (Noctuidae).

RESULTS AND DISCUSSION

Crude methanolic extracts from leaves or twigs of *A. duppereana* exhibited significant insecticidal activity towards neonate larvae of *S. littoralis*. When both extracts were incorporated into artificial diets at an arbitrarily chosen concentration of 1000 ppm and offered to the larvae in a chronic feeding bioassay over a period of 6 days, the twig extract was found to cause complete larval mortality, whereas 40% of the larvae survived on the diet containing the extract from leaves (data not shown). Consequently, the twig extract was chosen for a bioassay-guided isolation of the active principles.

Repeated chromatographic separation of the crude extract resulted in the isolation of seven insecticidal compounds. Based on their spectral characteristics and on comparison with known data, compounds **1** and **2** (Fig. 1) were readily identified as rocaglamide and desmethylrocaglamide, respectively. Both compounds are known from other *Aglaia*, species including *A. elliptifolia* [12], *A. odorata* [10, 11], *A. argentea* and *A. forbesii* [13]. In addition to these known compounds, five other rocaglamide derivatives (**3**–**7**) (Fig. 1) were isolated from twigs of *A. duppereana*. Compounds **3**–**5** and **7** are new natural products.

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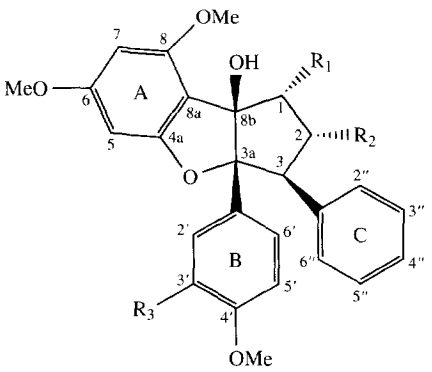
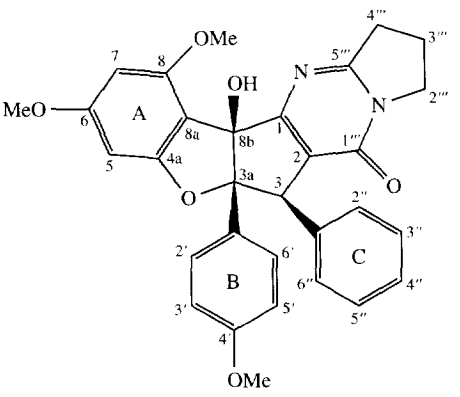


	R_1	R_2	R_3
1	OH	$CON(CH_3)_2$	H
2	OH	$CONHCH_3$	H
3	OH	$CON(CH_3)_2$	OH
4	OAc	$CON(CH_3)_2$	OH
5	OH	$CON(CH_3)_2$	OCH_3



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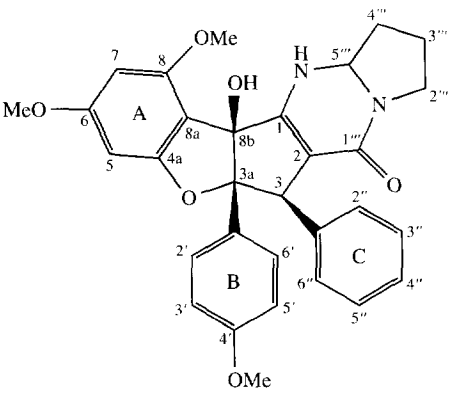

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Fig. 1. Structures of rocaglamide derivatives (**1**–**7**) isolated from *Aglaia duperreana*.

Compounds **3**–**5** are closely related to rocaglamide (**1**) and differ from it only in the nature of the substituents at C-1 or C-3′, respectively. Compound **3** proved to be the new C-3′-hydroxy derivative of rocaglamide (**1**), as is evident from its M_r of 521, which is 16 mu higher than that of rocaglamide (**1**), and from inspection of the ^1H and ^{13}C NMR spectra which allowed assignment of the hydroxyl substituent at C-3′ (Table 1). Compound **4** was identified as a new *O*-acetyl derivative of rocaglamide (**1**). The presence of an acetate substituent in compound **4** was evident from its mass spectrum. The $[M]^+$ of compound **4** was observed at *m/z* 563, which is 42 mu higher than that of compound **3**. Furthermore, the mass spectrum of compound **4** revealed a characteristic fragment at *m/z* 503, which is indicative of the loss of acetic acid. Assignment of the acetate unit at C-1 was clear from inspection of the ^1H NMR spectrum of compound **4** (Table 1). The resonance of H-1 (6.08 ppm) exhibited a large downfield shift compared with the corresponding signal in compound **1** (5.01 ppm). Compound **5** was the new C-3′ methoxyl derivative of rocaglamide (**1**) from its spectral data (Table 1).

Comparison of the mass spectra of compounds **1**–**5** revealed a characteristic pair of fragments in the range *m/z* 300–343, which proved to be indicative of the substitution pattern of ring B. The mass spectra of compounds **1** and **2**, which are both characterized by a C-4′ methoxylated B-ring showed a pair of fragments at *m/z* 300 and *m/z* 313, respectively. In the mass spectra of compounds **3** and **4**, which both have an extra hydroxyl substituent at C-3′, the respective fragments shifted to *m/z* 316 and *m/z* 329. In compound **5**, which has a C-3′, 4′ methoxylated B-ring, the characteristic fragments were observed at *m/z* 330 and *m/z* 343, respectively. Plausible structures for the ions *m/z* 316 (high-resolution EI-mass spectrum found: 316.09387, calc.: 316.094679) and *m/z* 329 (high-resolution EI-mass spectrum found: 329.10334, calc.: 329.102504) arising from fragmentation of compound **3** are given in Fig. 2.

The absolute configuration of rocaglamide (**1**) had previously been established through synthesis [14]. The optical rotation of **1** isolated from *A. duppereana* was $[\alpha]_D^{20}$ −89.0° (c = 0.17, $CHCl_3$) which is virtually identical to the reported optical rotation of the synthetic product $[\alpha]_D^{20}$ −88.8° (c = 1.03, $CHCl_3$) and very similar to the optical rotation of rocaglamide previously isolated from *A. odorata* $[\alpha]_D^{20}$ −93° (c = 1.88, $CHCl_3$) [11]. Hence, the absolute configuration of rocaglamide (**1**) from *A. duppereana* is as depicted in Fig. 1. The absolute configuration of the rocaglamide derivatives **2**–**5** which have the same chromophore as **1** (Fig. 1) was deduced by comparison of the respective CD spectra with the CD spectrum of compound **1**. All spectra were very similar, with a prominent negative

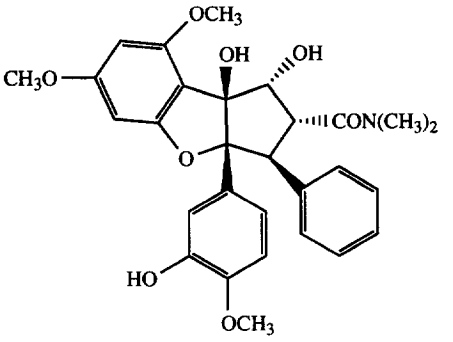
Table 1. ^{1}H and ^{13}C NMR data for compounds **1–7**

	1	**2**	**3***	**4**	**5**	**6***	**7***
^{1}H-NMR							
1	5.01 (*d*, 6.8)	4.81 (*d*, 6.0)	4.99 (*d*, 6.9)	6.08 (*m*)* 2nd order	5.02 (*d*, 6.9)		
2B	4.17 (*dd*, 6.8, 13.6)	3.85 (*dd*, 6.0, 14.2)	4.18 (*dd*, 6.9, 13.6)	4.35 (*m*)* 2nd order	4.14 (*dd*, 6.9, 13.5)		
3	4.41 (*d*, 13.6)	4.32 (*d*, 143)	4.39 (*d*, 13.6)	4.35 (*m*)* 2nd order	4.41 (*d*, 13.5)	4.49 (*s*)	4.37 (*s*)
5	6.35 (*d*, 1.9)	6.32 (*d*, 2.0)	6.34 (*d*, 2.0)	6.31 (*d*, 1.9)	6.37 (*d*, 1.9)	6.33 (*d*, 2.0)	6.37 (*d*, 2.0)
7	6.23 (*d*, 2.0)	6.21 (*d*, 1.9)	6.23 (*d*, 2.0)	6.70 (*d*, 1.9)	6.24 (*d*, 2.0)	6.17 (*d*, 2.0)	6.21 (*d*, 1.9)
2′	7.17 (*m*)	7.18 (*m*)	6.79 (*d*, 1.9)	6.83 (*d*, 2.1)	6.66 (*d*, 2.1)	7.08 ("*d*", 9.0)	7.18 ("*d*", 9.0)
3′	6.69 (*d*, 9.0)	6.66 (*d*, 9.1)	–	–	–	5.56 ("*d*", 9.0)	6.58 ("*d*", 9.0)
5′	6.69 (*d*, 9.0)	6.66 (*d*, 9.1)	6.69 (*d*, 8.5)	6.68 (*d*, 8.6)	6.80 (*d*, 8.5)	5.56 ("*d*", 9.0)	6.58 ("*d*", 9.0)
6′	7.17 (*m*)	7.18 (*m*)	6.72 (*dd*, 2.0, 8.6)	6.77 (*dd*, 2.2, 8.5)	6.96 (*dd*, 2.0, 8.4)	7.08 ("*d*", 9.0)	7.18 ("*d*", 9.0)
2″	6.91 (*m*)	6.98 (*m*)	6.91 (*m*)	6.96 (*m*)	6.90 (*m*)	6.87 (*m*)	7.10 (*m*)
3″	7.05 (*m*)	7.05 (*m*)	7.05 (*m*)	6.90 (*m*)	7.05 (*m*)	7.03 (*m*)	7.04 (*m*)
4″	7.05 (*m*)	7.05 (*m*)	7.05 (*m*)	6.90 (*m*)	7.05 (*m*)	7.03 (*m*)	6.94 (*m*)
5″	7.05 (*m*)	7.05 (*m*)	7.05 (*m*)	6.90 (*m*)	7.05 (*m*)	7.03 (*m*)	7.04 (*m*)
6″	6.91 (*m*)	6.98 (*m*)	6.91 (*m*)	6.96 (*m*)	6.90 (*m*)	6.87 (*m*)	7.10 (*m*)
OMe-6	3.87 (*s*)	3.86 (*s*)	3.87 (*s*)	3.87 (*s*)	3.87 (*s*)	3.84 (*s*)	3.87 (*s*)
OMe-8	3.89 (*s*)	3.88 (*s*)	3.89 (*s*)	3.78 (*s*)	3.90 (*s*)	3.84 (*s*)	3.90 (*s*)
OMe-3′					3.59 (*s*)		
OMe-4′	3.71 (*s*)	3.70 (*s*)	3.77 (*s*)	3.37 (*s*)	3.77 (*s*)	3.67 (*s*)	3.67 (*s*)
N-Me	2.92 (*s*); 3.39 (*s*)	2.69 (*s*)	3.39 (*s*); 2.92 (*s*)	2.84 (*s*); 3.42 (*s*)	2.92 (*s*); 3.38 (*s*)		
2‴A						4.15 (*m*)	3.65 (*m*)
2‴B						4.09 (*m*)	3.44 (*m*)
3‴A						2.38 (*m*)	2.12 (*m*)
3‴B						2.33 (*m*)	2.07 (*m*)
4‴A						3.33 (*m*)	2.57 (*m*)
4‴B						3.23 (*m*)	2.27 (*m*)
5‴							5.26 (*dd*, 6.0, 6.0)
CO-CH_3				1.86 (*s*)			

Table 1. *Continued.*

	1	**2**	**3***	**4**	**5**	**6***	**7***
^{13}C-NMR							
1	79.8	80.7	79.9	79.3	79.9	161.0	160.5
2	37.4	52.6	37.4	49.6	37.4	90.4	90.1
3	57.0	56.7	57.0	58.2	56.9	58.4	58.5
3a	102.6	102.7	102.3	102.2	102.3	106.1	106.5
5	90.3	90.0	90.3	89.5	90.4	93.5	90.2
7	93.2	93.0	93.2	92.7	93.3	90.0	93.4
8a	109.5	109.3	109.7	108.5	109.7	109.1	109.8
8b	95.2	95.1	95.2	94.0	95.2	121.5	103.4
1′	129.4	129.5	130.1	130.3	130.1	128.8	129.5
2′	130.2	130.2	116.7	116.7	114.4	129.9	129.6
3′	113.3	113.1	146.1	146.0	149.1	113.0	112.9
5′	113.3	113.3	111.3	111.3	111.4	113.0	112.9
6′	130.2	130.2	120.7	120.7	122.0	129.9	129.6
1″	139.8	139.1	139.7	139.1	139.9	138.2	140.7
2″, 6″	129.1	129.3	129.1	129.0	128.5	130.5	129.8
3″, 5″	128.4	128.5	128.4	128.6	127.2	128.3	128.2
4″	127.1	127.3	127.1	127.2	127.2	127.4	126.8
4′,8,4a,6	159.8	159.8	147.8	147.8	149.4	160.0	159.7
	159.2	159.3	159.2	159.7	159.5	159.8	159.4
	162.2	162.3	162.2	161.9	162.1	162.2	162.1
	165.3	165.2	165.3	165.4	165.4	165.3	165.5
1‴(CO)	171.7	173.3	171.8	171.3	171.7	169.7	162.2
2‴						48.1	45.3
3‴						20.3	24.0
4‴						33.4	32.9
5‴						168.0	72.0
ArOMe	56.1, 56.1, 55.5	56.1, 56.0, 55.4	56.2, 56.2, 56.1	56.2, 56.1, 55.8	56.5, 56.3, 56.1, 56.1	56.1, 55.7, 55.4	56.1, 55.8, 55.4
N-Me	36.0, 30.7	26.33	36.0, 29.5	37.7, 36.0	36.0, 30.7		
OCO-CH$_3$				170.4			
OCO-CH$_3$				20.8			

*Unambiguous assignments of both the ^{1}H and ^{13}C signals followed from the two-dimensional ^{1}H COSY, and ^{1}H-inverse detected one-bond and multiple bond ^{13}C–^{1}H correlations.



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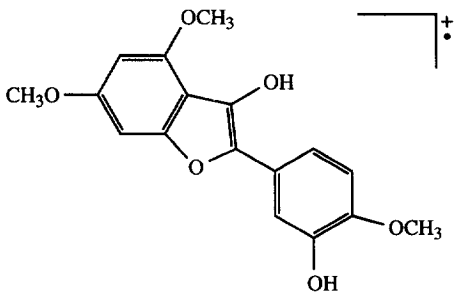
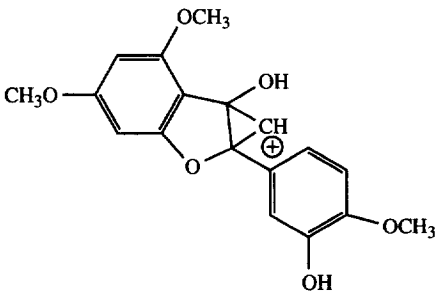


m/z 316



m/z 329

Fig. 2. Plausible structures of ions *m/z* 329 and 316 arising from fragmentation of compound **3** under EI-mass spectrometry.

Cotton effect between 217 and 220 nm as the most characteristic feature.

In addition to compounds **1**–**5**, two further metabolites (**6** and **7**; Fig. 1) were isolated which proved to be unusual rocaglamide derivatives featuring a pyrimidone unit. Based on its mass, and also ^{1}H and ^{13}C NMR spectra, compound **6** was found to be identical to a secondary metabolite recently isolated from roots of *A. odorata* [4]. Compound **7** is a new dihydro derivative of **6**. In the ^{13}C NMR spectrum of compound **7**, the signal for C-5‴ was observed at 72.0 ppm compared with 168.0 ppm in the ^{13}C NMR spectrum of compound **6** (Table 1); the signal for H-5‴ was split into a double-doublet due to geminal coupling with H-4‴ A and B, respectively (Table 1).

The CD spectra of compounds **6** and **7** are similar to those of the rocaglamide derivatives **1**–**5** with regard to the prominent negative Cotton effect between 217 and 220 nm, so it is feasible that they should have the same absolute configurations at C-3, C-3a and C-8b, as in the case of compounds **1**–**5**. However, it must be taken into consideration that the influence of the two missing stereocentres C-1 and C-2 and that of the additional stereocentre at C-5‴ of compound **7** is difficult to judge. Furthermore, the additional heterocyclic chromophors might exert an additional influence that is hard to estimate. For these reasons, the CD spectra of compounds **6** and **7** are not unambiguously comparable with those of **1**–**5**. Conversely, the UV spectra of these two subclasses of compounds are closely related, so that it is highly probable that compounds **6** and **7** show the depicted absolute configurations; this, however, remains to be confirmed. Thus, the structure of compound **6** (Fig. 1) represents only one of the two possible enantiomers, albeit the more probable. The same can be stated for compound **7**, with the additional problem of an unknown configuration at C-5‴.

All rocaglamide derivatives isolated from *A. duperreana* (**1**–**7**) proved to be insecticidal to neonate larvae of *S. littoralis* following incorporation into artificial diets at an arbitrarily chosen concentration of 100 ppm. At this concentration, which was routinely employed for bioassay-guided fractionation, all compounds caused complete mortality of the larvae after 6 days of exposure (data not shown). In a subsequent experiment each compound was tested for insecticidal activity at a range of concentrations. The well-known insecticidal compound, azadirachtin from *Azadirachta indica*, was included in these experiments as a positive control. The LC_{50} of each compound was calculated by probit analysis of the dose-response curves (Table 2). With the exception of the acetylated derivative **4**, all rocaglamide derivatives analysed exhibited comparable insecticidal activity with LC_{50} values ranging from 0.9 to 1.6 ppm; they are, therefore, essentially equitoxic to the powerful natural insecticide, azadirachtin, which exhibited a LC_{50} of

Table 2. LC_{50} and EC_{50} values of insecticidal rocaglamide derivatives **1**–**7** and of azadirachtin towards neonate larvae of *Spodoptera littoralis*

Compound	LC_{50} (ppm)	EC_{50} (ppm)
1	0.9	0.08
2	1.3	0.27
3	1.5	0.21
4	8.0	0.52
5	1.0	0.09
6	1.1	0.20
7	1.6	0.40
Azadirachtin	0.9	0.04

Chronic feeding experiment: neonate larvae of *S. littoralis* ($n = 20$) were released on diet spiked with various concentrations of the analysed compounds (0.01–15 ppm). After 6 days of exposure, survival and weight of the surviving larvae were measured and compared with controls that had been exposed to diet treated with solvent (MeOH) only. From the dose–response curves (means of three independent experiments) obtained, the respective LC_{50} and EC_{50} values were calculated by probit analysis.

0.9 ppm (Table 2). The EC_{50} values of the rocaglamide derivatives **1**–**3** and **5**–**7** ranged from 0.08 to 0.52 ppm compared with an EC_{50} of 0.04 ppm for azadirachtin (Table 2). The results obtained in the present work, as well as that in previous studies [10, 11] demonstrates the potent insecticidal activity of rocaglamide derivatives. The reduction of insecticidal activity caused by the presence of an acetyl substituent at C-1 of compound **4** instead of a hydroxyl substituent as in **1**–**3** or **5**, indicates the first structure–activity relationship in this group of powerful natural insecticides.

EXPERIMENTAL

Isolation and identification of compounds. Plant material was collected in Vietnam (near Ho-Chi-Minh-City) in April 1995. A voucher specimen is on file in the J.-v.-Sachs-Institut für Biowissenschaften, Universität Würzburg. Air-dried twigs (1 kg) were ground and exhaustively extracted with MeOH. Following evapn of solvent, the extract was partitioned between H_2O–hexane, H_2O–EtOAc and H_2O–*n*-BuOH (H_2O satd). Each fr. obtained was submitted to a bioassay with neonate larvae (see below). In this bioassay, the insecticidal activity was found to reside in the EtOAc fr. Bioassay-guided fractionation of this fr. was achieved through repeated chromatographic sepn employing silica gel (Merck) (mobile phase: CH_2Cl_2–*iso*PrOH, 9:1) and Sephadex LH-20 (mobile phase: Me_2CO). Final purification was obtained using RP-18 lobar columns (Merck) (mobile phase: 70% aq. MeOH). Frs were monitored by TLC on silica gel (F_{254}) (Merck) (mobile phase: CH_2Cl_2–*iso* PrOH, 9:1). Rocaglamide derivatives were detected by their dark absorbance under UV (254 nm) or after spraying with anisaldehyde reagent. Yields were: **1**, 12.6 mg, **2**, 11.7 mg, **3**, 5.7 mg, **4**, 5.0 mg, **5**, 2.3 mg, **6**, 8.5 mg and **7** 4.0 mg.

^1H and ^{13}C NMR spectra were recorded in CD_3OD on Bruker AM 300 or ARX 400 NMR spectrometers. EI-MS (70 eV) were obtained by direct inlet. MR data were determined by peak matching at a resolution of *ca* 10000 (10% valley). The structures of **1**–**7** were determined from 1D (^1H and ^{13}C) and 2D (COSY, ^1H-detected direct) and long-range ^{13}C–^1H correlations, and by comparison with published data [10–14].

Experiments with insects. Larvae of *S. littoralis* were from a laboratory colony reared on an artificial diet under controlled conditions [15]. Feeding studies were conducted with neonate larvae ($n = 20$ for each treatment). Neonate larvae were kept on diets containing extracts or compounds under study. After 6 days, survival and wts of 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 (Roth) was used as a positive control.

Compound **1**. $[\alpha]_D^{20}$ −89.0 (*c* 0.17, $CHCl_3$). CD: 217 nm (Δε −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).

Compound **2**. $[\alpha]_D^{20}$ −71.8 (*c* 0.41, $CHCl_3$). CD: 217 nm (Δε −19). EI–MS (*m/z*, rel. int.): 491 $[M]^+$ (16), 473 (12), 390 (38), 313 (58), 301 (90), 300 (100), 285 (50), 181 (44), 162 (92), 135 828), 131 (22).

Compound **3**. $[\alpha]_D^{20}$ −89.7 (*c* 0.21, $CHCl_3$). CD: 217 nm (Δε −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).

Compound **4**. $[\alpha]_D^{20}$ −83.3 (*c* 0.45, $CHCl_3$). CD: 217 nm (Δε −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).

Compound **5**. $[\alpha]_D^{20}$ −64.6 (*c* 0.18, $CHCl_3$). CD: 218 nm (Δε −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).

Compound **6**. $[\alpha]_D^{20}$ −50.1 (*c* 0.41, $CHCl_3$). CD: 219 nm (Δε −20), 242 nm (Δε +10), 280 nm (Δε +2). EI–MS (*m/z*, rel. int.): 524 $[M]^-$ (81), 506 (20), 389 (68), 370 (100), 343 (28), 313 (33), 135 (38).

Compound **7**. $[\alpha]_D^{20}$ +45.7 (*c* 0.19, $CHCl_3$). CD: 218 nm (Δε −25), 240 nm (Δε +2), 250 nm (Δε −2), 300 nm (Δε +10). EI–MS (*m/z*, rel. int.): 526 $[M]^+$ (6), 508 (100), 370 (16), 300 (22), 285 (16), 135 (16).

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