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Cornutins C–L, *neo*-clerodane-type diterpenoids from *Cornutia grandifolia* var. *intermedia*☆

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Abstract

Ten novel *neo*-clerodane diterpenoids, named cornutins C–L, have been isolated from the leaves of *Cornutia grandifolia* var. *intermedia*. Their structures have been elucidated by detailed spectroscopic analysis. In addition, the in vitro antiplasmodial activity of four isolated compounds (cornutin C–F) has been evaluated, revealing only a marginal activity.

Keywords: *Cornutia grandifolia* var. *intermedia*; Verbenaceae; Cornutins; *Neo*-clerodane diterpenoids; *Plasmodium falciparum*

1. Introduction

The neotropical genus *Cornutia* belonging to the family Verbenaceae comprises about 25 species. *Cornutia grandifolia* var. *intermedia* Moldenke is a shrub or small tree with large many-flowered inflorescences and blue or violet flowers. The species is native to Central American rainforests from Guatemala to Panama (Moldenke, 1973). It is used as a remedy against fever in Panama (Gupta et al., 1986). Earlier investigations of the leaves of *C. grandifolia* yielded the cornutins A and B, *neo*-clerodane-type diterpenoids with repellent activity against leafcutter ants (Chen et al., 1992).

During our ongoing investigations on antiplasmodial plant species from Central America, we isolated ten novel diterpenoids, named cornutins C–L (**1**–**10**), from the leaves of *C. grandifolia* var. *intermedia*.

2. Results and discussion

Ground, dried leaves of *C. grandifolia* var. *intermedia* were extracted at room temperature with a mixture of petrolether–EtOAc (1:1). After evaporation of the solvent, the oily residue was fractionated by MPLC over RP-18 material with H_2O–MeOH mixtures and MeOH to yield 16 fractions. Successive chromatographic purification finally led to the isolation of ten novel diterpenoids (**1**–**10**).

Compound **1** displayed a molecular ion peak at *m*/*z* 366 in the EIMS, as well as characteristic fragments at *m*/*z* 348 [M–H_2O] and *m*/*z* 97. Its molecular formula was determined as $C_{20}H_{30}O_6$ (HREIMS). The ^1H and ^{13}C NMR spectra revealed the presence of a β-substituted furan moiety (Tables 1 and 2). One of the furan protons (δ 6.33, *br s*) showed HMBC correlations to a hydroxymethine carbon atom at δ 63.6. The fragment at *m*/*z* 97 in the EIMS could thus be explained by the loss of a furan-CHOH-moiety from the molecule. The corresponding proton of the hydroxymethine residue (δ 4.82, *dd*, $J = 9.5$; 2.0 Hz) was coupled to a methylene group at δ 2.51 (1H, *dd*, $J = 9.5$; 16.0 Hz) and δ 1.69 (1H, *dd*, $J = 16.0$; 2.0 Hz). This methylene group showed long-range correlations to four further carbon atoms:

☆ Part 10 in the series "Herbal remedies traditionally used against malaria", for part 9 see Jenett-Siems et al. (2003).

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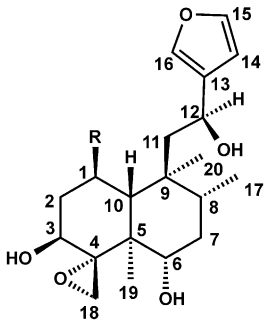

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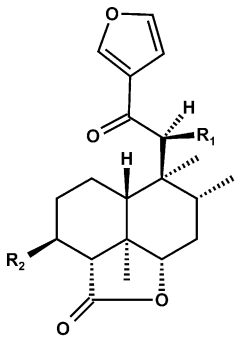
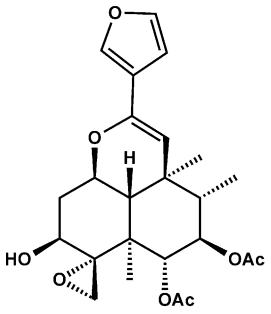
2 R = OAc



3 R_1 = OH, R_2 = H
5 R_1 = H, R_2 = OAc
6 R_1 = OAc, R_2 = H
7 R_1 = H, R_2 = H



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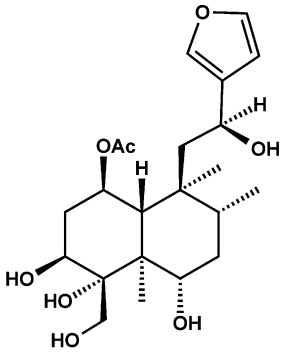

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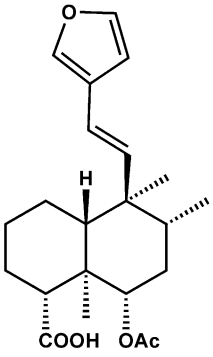

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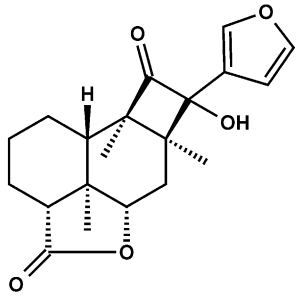

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one quaternary carbon at δ 40.3, two methine carbons at δ 51.7 and 36.7 and one methyl group at δ 17.9, thus evidencing a branching. The methyl group had to be attached to the quaternary carbon. One of the methine carbons (δ 36.7) bore an additional methyl group (δ 0.86, *d*, $J = 6.5$ Hz). These structural features in combination with the molecular formula led to the assumption that **1** had to be a furanoid clerodane-type diterpenoid. This skeleton normally possesses two further methyl groups in position 4 and 5. The ^{1}H NMR of **1** displayed only one further methyl singlet at δ 1.22 and two doublets at δ 3.18 and 3.36 ($J = 3.5$ Hz) characteristic for an exocyclic epoxide moiety. Thus the C-4 methyl group had to be oxidised. Finally the ^{1}H NMR showed three additional signals in the downfield region belonging to three further hydroxymethine protons. As all of them displayed at least one large coupling constant ($J = 11.0$–12.0 Hz), they had to be in an axial position. HMBC correlations (Table 3) allowed the assignment of the hydroxy groups to the carbons 1, 3, and 6. In analogy to the formerly isolated cornutins (Chen et al., 1992), **1** was named cornutin C.

Compound **2** (cornutin D) showed a molecular ion peak at *m*/*z* 408 in the EIMS, and its molecular formula of $C_{22}H_{32}O_7$ (HREIMS) suggested it to be an acetyl derivative of **1**. The ^{1}H NMR spectrum (Table 1) indeed was quite similar to that of **1**, displaying an additional signal at δ 2.12 (3H, *s*) corresponding to the acetyl moiety. Regarding the four hydroxymethine signals, that of H-1 was shifted downfield (δ 5.46, *td*, $J = 4.5$; 11.0 Hz), indicating the position of the acetyl residue.

Compound **3** again showed the typical signals of a β-substituted furan moiety in the ^{1}H NMR spectrum (Table 1), but in comparison to the former compounds, the signal for H-16 (δ 8.08, *br s*) was shifted downfield. This clearly pointed to a carbonyl group in position 12, which was supported by a signal at δ 201.2 in the ^{13}C NMR (Table 2) spectrum. Further evidence yielded the EIMS showing a fragment at *m*/*z* 95 with a molecular composition of $C_5H_3O_2$ (HREIMS). As in **1** and **2** the ^{1}H NMR spectrum displayed three methyl groups but no epoxide signals. Instead, in the ^{13}C NMR spectrum an additional signal at δ 180.2 was observed, suggesting C-18 to be oxidised to a carboxyl group. Finally, the ^{1}H NMR showed two hydroxymethine signals at δ 4.38 (1H, *s*) and δ 3.78 (1H, *dd*, $J = 4.5$, 12.5 Hz). The singlet possessed long-range correlations to the methyl group at δ 18.3 as well as to the carbonyl moiety at δ 201.2, therefore it had to be H-11. Thus, compound **3** is characterised by an unusual oxidised side-chain which was

Table 1
^{1}H NMR spectroscopic data for compounds **1**–**10** (400 MHz, $CDCl_3$)

H	1	2[a]	3	4	5	6	7	8[a]	9	10
1	4.22 *td* (11.0; 4.5)	5.46 *td* (11.0; 4.5)	1.65 *m* 1.45 *m*	4.51 *td* (11.0; 4.5)	1.76 *m*	2.04 *m* 1.51 *m*	1.35–1.52 *m*	5.33 *dt* (5.0; 11.5)	1.40–1.55 *m*	1.60 *m*
2	2.38 *dt* (12.0; 4.5) 1.40 *m*	2.36 *dt* (12.0; 4.5) 1.52 *m*	1.92 *m* 1.23 *m*	2.73 *dt* (12.0; 4.5) 1.63 *m*	2.23 *m* 1.32 *m*	1.95 *m* 1.30 *m*	1.35–1.52 *m* 1.25 *m*	1.77–1.84 *m* 2.14 *m*	1.27 *m* 1.68–1.82 *m*	2.05 *m* 1.28 *m*
3	4.11 *dd* (12.0; 5.0)	4.15 *dd* (12.0; 5.0)	1.75 *m* 1.43 *m*	4.14 *dd* (12.5; 4.5)	5.23 *dt* (10.5; 5.0)	1.82 *m*	1.82 *m* 1.95 *m*	3.96 *dd* (13.0; 5.5)	1.60 *m* 1.68–1.82 *m*	1.50 *m* 1.80 *m*
4			1.95 *m*		2.93 *d* (10.5)	1.97 *dd* (12.0; 4.0)	2.02 *dd* (10.5; 3.5)		2.14 *dd* (12.5; 3.0)	2.13 *dd* (12.0; 3.5)
6	3.69 *dd* (11.0; 4.5)	3.48 *dd* (11.0; 4.0)	3.78 *dd* (12.5; 4.5)	4.84 *d* (10.0)	4.21 *dd* (12.5; 4.0)	3.69 *dd* (12.0; 3.5)	3.94 *dd* (12.5; 4.0)	3.91 *dd* (11.0; 4.0)	4.70 *dd* (11.0; 4.0)	4.83 *dd* (12.0; 6.0)
7	1.52 *m*	1.52 *m*	1.89 *m* 1.68 *m*	5.12 *dd* (11.0; 10.0)	1.98 *dt* (12.5; 4.0) 1.76 *m*	1.78 *m* 1.93 *m*	1.82 *m* 1.95 *m*	1.26 *m* 1.48–1.54 *m*	1.40–1.55 *m* 1.68–1.82 *m*	2.60 *dd* (12.0; 6.0) 1.75 *t* (12.0)
8	1.83 *m*	1.60 *m*	2.10 *m*	1.63 *m*	2.56 *m*	1.93 *m*	2.28 *m*	1.48–1.54 *m*	1.60 *m*	
10	1.96 *d* (11.0)	2.27 *d* (11.0)	1.83 *dd* (12.0; 4.0)	1.65 *d* (11.0)	2.29 *dd* (12.0; 4.0)	1.67 *dd* (12.0, 3.0)	1.99 *dd* (12.0; 4.0)	2.70 *d* (11.5)	1.07 *dd* (12.0; 2.5)	2.26 *dd* (12.0; 4.5)
11	2.51 *dd* (16.0; 9.5) 1.69 *dd* (16.0; 2.0)	2.10 *m* 1.80 *dd* (15.0; 6.5)	4.38 *s*	5.08 *s*	2.98 *d* (19.5) 2.85 *d* (19.5)	5.43 *s*	2.69 *s*	1.77–1.84 *m* 2.08 *dd* (14.5; 6.0)	5.48 *d* (16.0)	
12	4.82 *dd* (2.0; 9.5)	4.92 *dd* (6.5)						4.95 *dd* (7.0; 6.0)	6.08 *d* (16.0)	
14	6.33 *br s*	6.46 *br s*	6.80 *br s*	6.42 *br s*	6.74 *br s*	6.80 *br s*	6.74 *br s*	6.47 *d* (1.5)	6.51 *d* (1.0)	6.37 *br s*
15	7.41 *t* (1.5)	7.46 *t* (1.5)	7.49 *t* (1.5)	7.35 *t* (1,5)	7.45 *t* (1.5)	7.46 *t* (1.5)	7.44 *t* (1.5)	7.45 *t* (1.5)	7.36 *t* (1.0)	7.43 *t* (1.5)
16	7.38 *br s*	7.49 *br s*	8.08 *br s*	7.53 *br s*	7.99 *br s*	8.14 *br s*	8.00 *br s*	7.53 *br s*	7.37 *br s*	7.46 *br s*
17	0.86 *d* (6.5)	0.78 *d* (7.0)	0.89 *d* (6.5)	0.96 *d* (6.5)	0.97 *d* (7.0)	1.08 *d* (6.5)	1.01 *d* (7.0)	0.76 *d* (7.0)	0.75 *d* (7.0)	0.95 *s*
18	3.36 *d* (3.5) 3.18 *d* (3.5)	3.32 *d* (3.5) 3.22 *d* (3.5)		3.22 *d* (3.5) 2.98 *d* (3.5)				4.10 *d* (11.5) 4.05 *d* (11.5)		
19	1.22 *s*	1.23 *s*	0.88 *s*	1.35 *s*	0.93 *s*	0.88 *s*	0.87 *s*	0.77 *s*	1.21 *s*	0.78 *s*
20	0.93 *s*	0.78 *s*	0.98 *s*	1.15 *s*	0.95 *s*	0.99 *s*	0.89 *s*	1.14 *s*	0.92 *s*	1.12 *s*
2′		2.12 *s*		2.00 *s*	1.81 *s*	2.14 *s*		2.11 *s*	1.96 *s*	
2″				2.03 *s*						

[a] In CD_3OD.

supported by a fragment at m/z 126 ($C_6H_6O_3$) in the EIMS. The second hydroxy group had to be in position 6 according to the HMBC correlations and regarding the coupling constants of H-6, the hydroxy group again had to be in an equatorial position. The EIMS and FABMS spectra of compound **3**, which we named cornutin E, showed a molecular ion peak at m/z 346 ($C_{20}H_{26}O_5$), suggesting the formation of a lactone ring between the carboxyl group at C-18 and the hydroxy group at position 6 which indeed is a common feature of *neo*-clerodanes from the families Lamiaceae or Verbenaceae. This was supported by the IR spectrum showing a typical lactone band at 1764 cm^{-1}. Nevertheless the chemical shift of H-6 (δ 3.78) raised some doubts because it should be found between δ 4.10 and 4.60 in case of lactonization (Kitagawa et al., 1994). Therefore, compound **3** was treated with acetic anhydride/pyridine 1:1, which only led to the acetylation of the C-11-OH (**3a**), thus proving the existence of a lactone ring.

A NOESY spectrum of **3** displayed correlations of the axial proton at C-6 to the protons in positions 4, 8, and 10, revealing the same relative stereochemistry as in the already isolated cornutins A and B (Chen et al., 1992). Thus we assume that for biogenetic reasons the whole series of isolated compounds represents *neo*-clerodane-type diterpenoids, although this has not been unambiguously proven. The orientation of the epoxide moiety in **1** could also be deduced from NOE experiments. Irradiation in the proton signal at δ 3.36 (H-18a) led to

Table 2
^{13}C NMR spectroscopic data for compounds **1**, **3**, **4**, and **6–10** (100 MHz, $CDCl_3$)

C	1	3	4	6	7	8[a]	9	10
1	68.8	22.6	68.6	22.1	20.5	71.8	21.9	22.6
2	45.6	27.1	38.6	25.9	26.0	38.5	25.9	26.1
3	63.9	20.4	62.6	19.3	19.4	71.8	25.9	18.9
4	72.4	56.9	68.9	55.8	55.9	79.6	55.8	53.5
5	42.2	44.1	40.6	42.7	40.9	40.4	41.0	42.0
6	75.0	89.0	75.4	86.8	87.7	75.2	81.1	85.0
7	34.8	32.4	74.0	30.9	30.8	36.0	32.0	30.5
8	36.7	36.0	44.0	36.2	37.8	35.7	39.0	46.1
9	40.3	46.7	35.6	44.1	43.0	46.9	43.0	65.2
10	51.7	47.8	50.2	46.8	47.1	45.5	53.2	44.3
11	47.4	82.9	104.0	82.1	47.0	48.2	141.5	213.2
12	63.6	201.2	143.3	192.3	194.5	64.5	119.1	89.6
13	132.7	128.6	122.3	127.9	129.5	131.9	124.0	121.7
14	109.4	110.3	107.2	109.0	108.6	109.8	108.0	108.4
15	144.6	144.7	143.2	144.2	144.4	144.6	143.7	140.4
16	139.4	150.8	139.6	147.4	146.9	140.5	140.0	143.8
17	16.1	18.3	10.2	17.2	16.2	16.2	17.9	25.1
18	47.6	180.2	46.3	177.4	177.7	62.6	n.o.	176.9
19	14.3	12.8	16.7	12.7	12.9	11.2	10.5	9.8
20	17.9	15.9	20.6	15.8	19.1	19.1	12.1	13.6
1′			170.0	170.3		172.2	170.5	
2′			20.8	20.8		21.9	21.1	
1″			170.6					
2″			20.7					

[a] In CD_3OD.

an enhancement of the signal of H-6, proving an axial position of the C-4-methyl group.

The compounds **5–7** all showed a downfield shift of H-16 in the ^{1}H NMR spectrum indicative of a carbonyl group in position 12 and possessed a lactone moiety, which was deduced from their IR spectra (**6** and **7**). Thus, they displayed similar structural features as **3**. In the EIMS of **6** a molecular ion peak at *m/z* 388 appeared, suggesting a molecular formula of $C_{22}H_{28}O_6$. These data led to the assumption that **6** had to be an acetylated derivative of **3**. Indeed, the ^{1}H NMR spectrum of **6** displayed a characteristic signal for an acetyl moiety (δ 2.14, 3H, *s*) and the signal of H-11 (δ 5.43, *s*) was shifted downfield compared to **3**, showing an esterification in this position, whereas the other spectroscopic data of the two compounds were quite similar. Thus, **6** (cornutin H) had to be the 11-acetyl derivative of **3**.

Compound **5** (cornutin G) possessed the same molecular composition as **6**. The ^{1}H NMR spectrum showed again two hydroxymethine groups. From decoupling experiments one of the signals was assigned to H-6 (4.21, *dd*, $J = 12.5$; 4.0 Hz), the chemical shift of which is indicative of a lactonization, whereas the other had to be H-3 (5.23, *dt*, $J = 5.0$; 10.5 Hz) which in this case had to bear the acetyl moiety. The isolated geminal protons at C-11 gave a pair of doublets at δ 2.98 ($J = 19.5$ Hz) and 2.85 ($J = 19.5$ Hz).

In the EIMS of **7** (cornutin I) a molecular ion peak at *m/z* 330 appeared, thus, compound **7** had to bear one hydroxy group less than **3**. The position of the remaining hydroxy function was deduced from decoupling experiments and could be assigned to C-6. Due to the IR band at 1779 cm^{-1} the C-6-OH again had to be lactonized.

Compound **4** showed a molecular ion peak at *m/z* 446 in the EIMS spectrum, characteristic fragments were observed at *m/z* 371 and 95. The latter pointed to a furan moiety attached to an oxygen-substituted side-chain as in **3**. The fragment at *m/z* 371 could be

Table 3
HMBC correlations of compounds 1, 3, 4, 9, and 10 (CD_3OD)

H	1	3	4	9	10
1	10	2, 10	9	n.o.	n.o.
2	1, 3, 4, 10	10	1, 3, 10	n.o.	n.o.
3	2, 4	4	1, 4	2, 4	n.o.
4		5, 6, 10		5, 19	5
6	4, 7, 19	4, 19	4, 7, 19, 1′	4, 5, 19, 1′	4, 19
7	6, 8, 9, 17	6, 8, 17		5, 6, 8	5, 6, 9, 8, 12, 17
8	17	7, 9, 17, 20	n.o.	7, 17	
10	1, 5, 9, 11, 19, 20	1	1, 5, 6, 8, 9,19, 20	4, 6	5, 6, 9, 11, 19, 20
11	8, 9, 10, 12, 20	8, 9, 12, 20	8, 9, 10, 12, 13	8, 12, 20	
12	9, 11, 13, 14, 16			9, 11, 14	
14	12, 13, 15, 16	13, 15, 16	13, 15, 16	13, 15, 16	13, 15
15	13, 16	13, 16	13, 16	13, 16	14, 16
16	14, 15	14, 15	13, 14, 15	13	13, 15
17	7, 8, 9	7, 8	7, 8, 9	7, 8, 9	7, 8, 9, 12
18	3, 4, 5		4		
19	4, 5, 6, 10	4, 5, 6, 10	4, 5, 6, 10	4, 5, 6, 10	4, 5, 6, 10
20	8, 9, 10, 11	8, 9, 11	8, 9, 10, 11	8, 9, 10, 11	8, 9, 10, 11
2′			1′	1′	
2″			1″		

n.o. not observed

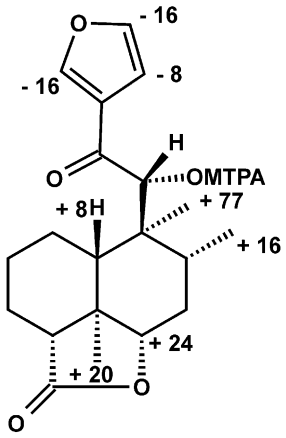

Fig. 1. Selected chemical shift differences between **3b** and **3c**. $\Delta\delta$ Values ($\Delta\delta = \delta_{(S)\text{-MTPA ester (3c)}} - \delta_{(R)\text{-MTPA ester (3b)}}$) are shown in Hz.

explained by the loss of one methyl group as well as acetic acid and indeed two signals typical for acetyl residues at δ 2.00 and 2.03 were present in the ^{1}H NMR (Table 1). In addition, it displayed signals for the furan moiety, three methyl groups, an epoxide sub-structure and five further downfield shifted proton signals. The singlet at δ 5.08 had to be due to an olefinic or acetalic proton, as the corresponding signal in the ^{13}C NMR spectrum could be found at δ 104.0. This proton possessed long-range correlations to one of the furan carbons as well as to a quaternary olefinic carbon at δ 143.3. The chemical shifts of the olefinic carbons hinted at an enolether sub-structure. Furthermore, the olefinic proton showed HMBC correlations to another quaternary carbon atom and a methine carbon at δ 50.2. Thus, it had to be H-11, whereas C-12 had to be substituted with the enolic oxygen. The methyl group at δ 0.96 (3 H, *d*, $J = 6.5$ Hz) displayed HMBC cross-peaks to an oxygen substituted carbon atom (δ 74.0). Therefore C-7 had to bear a hydroxy function. The chemical shift of the corresponding proton in the ^{1}H NMR spectrum (δ 5.12; *dd*, $J = 10.0$; 11.0 Hz) indicated an esterification with one of the acetyl moieties. The large coupling constants again proved an equatorial position of the hydroxy group. H-7 was coupled to a doublet at δ 4.84, which showed HMBC correlations to the second acetyl residue. The remaining two signals in the downfield region had to be H-1 and H-3 due to their coupling patterns. Their relative stereochemistry was analogous to the former compounds. In comparison to **1**, H-1 was shifted downfield, suggesting an enolether between C-12 and C-1. Thus, compound **4** (cornutin F) comprises an additional six-membered ring as the formerly isolated cornutin A (Chen et al., 1992), such an enolether is, to date unique within the *neo*-clerodane diterpenoids.

Compound **8** gave a fragment at *m*/*z* 408 in the EIMS, corresponding to a molecular formula of $C_{22}H_{32}O_7$ (HREIMS), which had to be due to the loss of water. The (−) FAB MS displayed a peak at 425 $[M-H]^-$. Thus, compared to compound **2**, a mass difference of 18 was observed. The ^{1}H NMR also showed similarities to that of **2**, revealing the same number and multiplicity of hydroxymethine protons but no characteristic epoxide protons. Instead, a pair of isolated geminal protons at δ 4.10 (*d*, $J = 11.5$ Hz) and 4.05 (*d*, $J = 11.5$ Hz) pointed to a 4,18-diol sub-structure. This was supported by the downfield shift of the corresponding carbons in the ^{13}C NMR spectrum (Table 2). Thus, **8** (cornutin J) had to be the 4,18-diol derivative of **2**.

Compound **9** displayed a molecular ion peak at *m*/*z* 374, corresponding to a molecular formula of $C_{22}H_{30}O_5$ (HREIMS). In addition to the characteristic signals for a furano-*neo*-clerodane, two olefinic signals at δ 5.48 (1H, *d*, $J = 16.0$ Hz) and 6.08 (1H, *d*, $J = 16.0$ Hz) were present in the ^{1}H NMR spectrum (Table 1), characteristic for a *trans*-configurated double bond. From the HMBC spectrum (Table 3), these protons were assigned to H-11 and H-12. Furthermore, the signal at δ 4.70 (1H, *dd* $J = 11.0$; 4.0 Hz) indicated the presence of an acylated hydroxy group. Indeed, the typical signals for an acetyl moiety were observed in the NMR spectra. The position of the hydroxy group was again deduced from the HMBC spectrum. Thus, the structure of **9** (cornutin K) was assigned.

Compound **10** possessed a molecular ion peak of *m*/*z* 344, corresponding to a molecular formula of $C_{20}H_{24}O_5$ (HREIMS). The ^{1}H NMR spectrum of **10** again showed the characteristic signals of a β-substituted furan ring system but interestingly three methyl singlets were present, indicating a substitution at C-8. In addition, the typical signal of the hydroxymethine proton at C-6 was observed. Compared to the other isolated compounds, it was notably shifted downfield (δ 4.83). As the NMR spectra did not show any acetyl moieties, this had to be due to formation of a lactone ring between the C-6 hydroxy group and the carboxy group of C-18 (IR band 1771 cm^{-1}). The ^{13}C NMR spectrum revealed the presence of a carboxy group (δ 176.9), a carbonyl group (δ 213.2) and a quaternary oxygen-substituted carbon atom (δ 89.6). The methyl singlet at δ 0.78 showed long-range correlations to H-6 and H-4 and could thus be identified as H-19. The singlet at δ 1.12 possessed HMBC-correlations to a methine carbon at δ 44.3, two quaternary carbon atoms (δ 46.1 and 65.2) and to the carbonyl group and was therefore assigned to H-20. The remaining singlet (δ 0.95) displayed HMBC cross-peaks to the same two quaternary carbon atoms, a methylene group (δ 30.5), and to the quaternary oxygen-substituted carbon atom at δ 89.6. Thus, C-11 should bear the carbonyl oxygen, whereas C-12 was connected to C-8 forming a four-membered carbon ring structure and in addition had to bear a tertiary hydroxy group. This rather unique *neo*-clerodane-type diterpenoid with a new type of carbon skeleton was named cornutin L (**10**).

Finally, the stereochemistry of the side-chain hydroxy groups (C-11 or C-12) remained to be established. Jiménez-Barbero (1993) proposed a method for the determination of the conformation of alcohol functions at the C-12 position of *neo*-clerodanes from *Teucrium* species based on NOE measurements. As a prerequisite the occurrence of a major rotamer around the C-11–C-12 bond in solution has to be proved by the existence of coupling constants between H-12 and the methylene protons at C-11 of higher than 8.5 Hz and smaller than 3.0 Hz, respectively. This is clearly the case in compound **1**. Thus, the observed NOE correlations between H-8 and H-12 are strongly indicative of a 12-*S*-configuration. In the case of the 12-*R*-isomer NOE correlations between H-12 and H-10 had to be expected. The same 12-*S*-configuration is assumed for the structurally related compounds **2** and **8**. Unfortunately the stereochemistry of the hydroxy group at C-12 in compound **10** could not be deduced from NOE experiments due to the lack of indicative correlations.

The absolute configuration of C-11 in **3** was determined by the advanced Mosher method (Ohtani et al., 1991). 11-*O*-(*R*)- and 11-*O*-(*S*)-Methoxy-(trifluoromethyl)phenylacetyl (MTPA) esters (**3b** and **3c**) were prepared and submitted to ^{1}H NMR spectroscopy. The $\Delta\delta$ values ($\Delta\delta = \delta_{(S)\text{-MTPA ester (3c)}} - \delta_{(R)\text{-MTPA ester (3b)}}$) of selected protons are shown in Fig. 1, indicating that the absolute configuration at C-11 should be *R*. The same configuration is assumed for the acetylated derivative **6**.

As *C. grandifolia* var. *intermedia* is traditionally used against fever in Panama, we evaluated the in vitro antiplasmodial properties of the cornutins C–F (**1**–**4**). Of these, only **1** and **2** possessed slight activity against two different strains of *P. falciparum* (poW, Dd2), with the acetylated compound **2** being the less active one [IC_{50} 23.1 μg/ml (56.6 μM, poW); 39.5 μg/ml (96.8 μM, Dd2)], whereas **1** showed IC_{50} values of 14.6 (36.9 μM, poW) and 21.5 μg/ml (58.7 μM, Dd2).

3. Experimental

3.1. General

^{1}H, ^{13}C, ^{1}H–^{1}H COSY, and ^{1}H–^{13}C COSY spectra were run in $CDCl_3$ solution on a Bruker AVANCE DPX 400 (400 MHz, TMS as internal standard). HMBC and NOESY spectra were obtained on a Bruker DRX 500 MHz spectrometer. EIMS were recorded on a Finigan MAT CH7A (70 eV), HRMS on a Finnigan MAT 711 (80 eV) and FAB-MS on a Finnigan MAT CH_5DF. Optical rotations were measured with a Perkin Elmer 241 MC. IR spectra were obtained on a Perkin Elmer 1420 spectrophotometer. Preparative column chromatography was performed on Si gel 60 (70–230 mesh, Merck). Preparative high performance liquid chromatography (HPLC) separation was performed on a Knauer pumping system with a Knauer variable wavelength detector (225 nm) equipped with a Knauer Nucleosil 300 C-18 column (10 μm, 22×250 mm).

3.2. Plant material

Aerial parts of *Cornutia grandifolia* var. *intermedia* were collected at the road from El Valle to La Meseta, Panama. The species was identified by Professor M. D. Correa A., Herbarium of the Universidad de Panama, Panama City, Panama, where voucher specimens (Florpan 2741, PMA) are deposited.

3.3. Extraction and isolation

Ground, dried leaves (400 g) were extracted 3× with 2 l petrolether/EtOAc 1:1 for 24 h each. After evaporation of the solvent, the oily residue (26 g) was fractionated by MPLC over RP-18 material with H_2O/MeOH mixtures (60:40; 50:50; 40:60) and MeOH to yield 16 fractions. Fraction 8 which was eluted with H_2O/MeOH 50:50 contained pure **1** (500 mg). Fraction 6, which was obtained with the same solvent mixture, was further purified by peparative HPLC (H_2O/MeOH 65:35→30:70 in 40 min) to give 10 mg of **2** (R_t = 22 min). Fraction 7, which also eluted with H_2O/MeOH 50:50 was separated by column chromatography on silica gel with $CHCl_3$ and $CHCl_3$/MeOH mixtures (98:2; 95:5; 90:10; 80:20; 70:30). Purification of fraction 7/6, which was obtained with $CHCl_3$/MeOH 90:10, by preparative HPLC (H_2O/MeOH 60:40→30:70 in 40 min) yielded 10 mg of **8** (R_t = 26 min). Fraction 10 eluted with H_2O/MeOH 40:60. After purification by prep. HPLC (H_2O/MeOH 50:50→20:80 in 40 min) **3** (R_t = 12 min) was obtained as white crystals (50 mg). The remaining solution was separated by prep. TLC ($CHCl_3$/EtOAc 95:5) and afforded again **3** (R_f = 0.41, 10 mg) and 3 mg of **5** (R_f = 0.48). Fraction 11, which was eluted with the same solvent mixture as 10, was fractionated by column chromatography on silica gel with cyclohexane/EtOAc mixtures of increasing polarity (90:10; 80:20; 70:30; 50:50; 30:70). Fraction 11/1, obtained with cyclohexane/EtOAc 90:10 contained 13 mg of **7**, whereas fraction 11/3, eluting with cyclohexane/EtOAc 70:30 contained pure **10** (15 mg). Fraction 11/4 which also eluted with cyclohexane/EtOAc 70:30 was purified by prep. TLC with $CHCl_3$/EtOAc 99:1 to yield **6** (R_f = 0.29, 10 mg). Fraction 12, which also was eluted with H_2O/MeOH 40:60, was further fractionated by column chromatography on silica gel with cyclohexane/EtOAc mixtures of increasing polarity (90:10; 80:20; 70:30; 50:50; 30:70). Fraction 12/6, which was eluted with cyclohexane/EtOAc 7:3 contained 20 mg of **4**. Fraction 13, which

was eluted with MeOH, was further fractionated by column chromatography on silica gel with $CHCl_3$/EtOAc mixtures of increasing polarity (98:2; 95:5; 90:10; 80:20). Fraction 13/4, which was eluted with $CHCl_3$/EtOAc 95:5 was purified by prep. HPLC (H_2O/MeOH 40:60→1:99 in 50 min) and yielded **9** (3 mg, $R_t = 32$ min).

3.4. Cornutin C (1)

Amorphous white solid. $[\alpha]_D^{20}$ −4° (*c* 0.12, MeOH); IR (KBr) ν_{max} cm^{-1}: 1458, 1155, 1020, 874; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Tables 1 and 2; EIMS (80 eV): *m/z* (rel. int) 366 $[M]^+$ (100), 348 (62), 252 (18), 234 (19), 97 (72), 95 (64); HREIMS: *m/z* 366.2042 (calc. for $C_{20}H_{30}O_6$, 366.2042).

3.5. Cornutin D (2)

Colourless oil. $[\alpha]_D^{20}$ −5° (*c* 0.08, MeOH); IR (KBr) ν_{max} cm^{-1}: 1730, 1242, 1158, 874; ^1H NMR (400 MHz, CD_3OD): Table 1; EIMS (80 eV): *m/z* (rel. int) 408 $[M]^+$ (19), 406 (16), 348 (82), 312 (32), 234 (25), 97 (100); HREIMS: *m/z* 408.2147 (calc. for $C_{22}H_{32}O_7$, 408.2148), 348.1936 (calc. for $C_{20}H_{28}O_5$, 348.1937), 97.0293 (calc. for $C_5H_5O_2$, 97.0290).

3.6. Cornutin E (3)

Colourless crystals. $[\alpha]_D^{20}$ −75° (*c* 0.30, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 1764, 1660, 1509, 1155, 926, 872; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Tables 1 and 2; EIMS (80 eV): *m/z* (rel. int) 346 $[M]^+$ (33), 328 (61), 313 (31), 221 (100), 126 (69), 95 (61); (−) FAB MS: *m/z* 345 $[M-H]^-$; HREIMS: *m/z* 346.1776 (calc. for $C_{20}H_{26}O_5$, 346.1780), 221.1573 (calc. for $C_{14}H_{21}O_2$, 221.1542), 95.0156 (calc. for $C_5H_3O_2$, 95.0133).

3.7. Cornutin F (4)

Colourless crystals. $[\alpha]_D^{20}$ +17° (*c* 0.34, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 1743, 1252, 1038, 874; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) see Tables 1 and 2; EIMS (80 eV): *m/z* (rel. int) 446 $[M]^+$ (38), 371 (86), 310 (16), 273 (13), 95 (91); HREIMS: *m/z* 446.1973 (calc. for $C_{24}H_{30}O_8$, 446.1940).

3.8. Cornutin G (5)

Colourless oil. $[\alpha]_D^{20}$ −2° (*c* 0.29, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): Table 1; EIMS (80 eV): *m/z* (rel. int) 388 $[M]^+$ (1), 328 (14), 218 (100), 126 (7), 110 (21), 95 (20); HREIMS: *m/z* 388.1883 (calc. for $C_{22}H_{28}O_6$, 388.1885).

3.9. Cornutin H (6)

Yellow crystals. $[\alpha]_D^{20}$ −10° (*c* 0.73, $CHCl_3$); IR (film) ν_{max} cm^{-1}: 1781, 1744, 1680, 1235, 1038, 872; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Tables 1 and 2; EIMS (80 eV): *m/z* (rel. int) 388 $[M]^+$ (1), 328 (13), 221 (100), 168 (82), 126 (81), 110 (12), 95 (42); HREIMS: *m/z* 388.1887 (calc. for $C_{22}H_{28}O_6$, 388.1885).

3.10. Cornutin I (7)

Colourless crystals. $[\alpha]_D^{20}$ +4° (*c* 0.31, $CHCl_3$); IR (film) ν_{max} cm^{-1}: 1779, 1768, 1261, 1040, 872; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Tables 1 and 2; EIMS (80 eV): *m/z* (rel. int) 330 $[M]^+$ (12), 221 (100), 110 (25), 95 (27); HREIMS: *m/z* 330.1831 (calc. for $C_{20}H_{26}O_4$, 330.1831).

3.11. Cornutin J (8)

Amorphous white solid. $[\alpha]_D^{20}$ +13° (*c* 0.35, MeOH); IR (film) ν_{max} cm^{-1}: 1243, 1021, 873; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Tables 1 and 2; EIMS (80 eV): *m/z* (rel. int) 408 $[M-H_2O]^+$ (4), 393 (2), 377 (62), 299 (100), 205 (82), 95 (33); (−) FAB MS: *m/z* 425 $[M-H]^-$; HREIMS: *m/z* 408.2147 (calc. for $C_{22}H_{32}O_7$, 408.2148).

3.12. Cornutin K (9)

Colourless oil. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Tables 1 and 2; EIMS (80 eV): *m/z* (rel. int) 374 $[M]^+$ (10), 330 (18), 312 (18), 221 (100), 121 (37), 95 (31); HREIMS: *m/z* 374.2093 (calc. for $C_{22}H_{30}O_5$, 374.2093).

3.13. Cornutin L (10)

Amorphous white solid. $[\alpha]_D^{20}$ +35° (*c* 0.39, MeOH); IR (film) ν_{max} cm^{-1}: 1771, 1237, 872; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): Tables 1 and 2; EIMS (80 eV): *m/z* (rel. int) 344 $[M]^+$ (11), 221 (100), 95 (78); HREIMS: *m/z* 344.1622 (calc. for $C_{20}H_{24}O_5$, 344.1624).

3.14. Acetylation of 3 and 7

A solution of the respective compound (10 mg) in a mixture of pyridine (0.8 ml) and acetic anhydride (0.8 ml) was allowed to stand at room temperature for 24 h. To the reaction mixture was added a small amount of water and it was acidified with HCl (10%). Extraction with $CHCl_3$ yielded **3a** and unchanged **7**, respectively.

3a: Colourless oil. ^1H NMR (400 MHz, $CDCl_3$): δ 0.81 (3H, *s*, H-19), 0.92 (3H, *s*, H-20), 1.01 (3H, *d*, $J = 6.5$ Hz, H-17), 1.22 (1H, *m*, H-2a), 1.45 (2H, *m*, H-1a/H-3a), 1.60

(1H, *dd*, *J* = 12.0; 3.0 Hz, H-10), 1.75 (2H, *m*, H-1b/H-7a), 1.83 (1H, *m*, H-7b), 1.88 (3H, *m*, H-4/H-8/H-2b), 1.98 (1H, *m*, H-3b), 2.07 (3H, *s*, Ac), 3.62 (1H, *dd*, *J* = 12.0; 3.5 Hz, H-6), 5.36 (1H, *s*, H-11), 6.73 (1H, *br s*, H-14), 7.39 (1H, *t*, *J* = 1.5 Hz, H-15), 8.07 (1H, *br s*, H-16).

3.15. 11-O-(R)- and 11-O-(S)-MTPA ester (*3b* and *3c*, respectively) of *3*

To a solution of **3** (6 mg) in CH_2Cl_2 (0.8 ml) was added successively DMAP (8.5 mg), Et_3N (3.5 μl), and (+)-MTPA chloride (6.3 μl) and the mixture was allowed to stand at room temperature for 17 h. *N*,*N*-Dimethyl-1,3-propanediamine (4.2 μl) was added, and the residue obtained after evaporation of the solvent was applied to preparative TLC (cyclohexane/EtOAc 1:1) to give pure (*R*)-MTPA ester **3b** (7.2 mg). In the same manner, **3c** (5.8 mg) was obtained from **3**.

3b: Colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (3H, *s*, H-19), 0.82 (3H, *s*, H-20), 1.03 (3H, *d*, *J* = 6.5 Hz, H-17), 1.12–1.30 (2H, *m*, H-2a/H-3a), 1.42 (1H, *dq*, *J* = 4.0; 12.5 Hz, H-1a), 1.52 (1H, *dd*, *J* = 12.0; 3.0 Hz, H-10), 1.65–1.95 (7H, *m*, H-1b/H-2b/H-3b/H-4/H-7/H-8), 3.53 (3H, *s*, OCH_3), 3.54 (1H, *dd*, *J* = 12.0; 3.5 Hz, H-6), 5.46 (1H, *s*, H-11), 6.80 (1H, *br s*, H-14), 7.44 (3H, *m*, H-5′/H-6′/H-7′), 7.49 (1H, *t*, *J* = 1.5 Hz, H-15), 7.59 (2H, *m*, H-4′/H-8′), 8.15 (1H, *br s*, H-16).

3c: Colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, *s*, H-19), 1.01 (3H, *s*, H-20), 1.07 (3H, *d*, *J* = 6.5 Hz, H-17), 1.22–1.32 (2H, *m*, H-2a/H-3a), 1.42 (1H, *dq*, *J* = 4.0; 12.5 Hz, H-1a), 1.54 (1H, *dd*, *J* = 12.0; 3.0 Hz, H-10), 1.60–1.98 (7H, *m*, H-1b/H-2b/H-3b/H-4/H-7/H-8), 3.54 (3H, *s*, OCH_3), 3.60 (1H, *dd*, *J* = 12.0; 3.5 Hz, H-6), 5.46 (1H, *s*, H-11), 6.78 (1H, *br s*, H-14), 7.41 (3H, *m*, H-5′/H-6′/H-7′), 7.45 (3H, *m*, H-15/H-4′/H-8′), 8.11 (1H, *br s*, H-16).

3.16. Cultivation of P. falciparum and in vitro bioassay

P. falciparum strain PoW (IC_{50} for chloroquine = 0.015 μM) and the clone Dd2 (IC_{50} = 0.18 μM) were maintained in continuous culture in human red blood cells as described by Trager and Jensen (1976). Substances were dissolved in DMSO (20 mg/ml) and diluted in medium to final concentrations between 50 and 0.78 μg/ml. The antiplasmodial assay, according to Desjardins et al. (1979) was analysed by means of a microculture radioisotope technique after 42 h as described in Kraft et al. (2000).

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