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Cytotoxic cardenolides and antibacterial terpenoids from *Crossopetalum gaumeri*

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Abstract

From the methanol extract of the roots of *Crossopetalum gaumeri*, four new highly cytotoxic cardenolides, securigenin-3β-*O*-β-6-deoxyguloside (**2**), 19-hydroxy-sarmentogenin-3β-*O*-β-6-deoxyguloside (**4**), sarmentogenin-3β-*O*-[α-allosyl-(1 → 4)-β-6-deoxyalloside] (**5**), and securigenin-3β-*O*-[α-allosyl-(1 → 4)-β-6-deoxyalloside] (**6**) were isolated. The dichloromethane extract afforded the new diterpene 3,15-dihydroxy-18-norabieta-3,8,11,13-tetraene (**7**) as well as the new triterpene 2,3,7-trihydroxy-6-oxo-1,3,5(10),7-tetraene-24-nor-friedelane-29-oic acid methylester (**11**). The new terpenoids lack cytotoxicity and the antibacterial activity is moderate to low.

Keywords: *Crossopetalum gaumeri*; Celastraceae; Cardenolides; Triterpenes; Diterpene; Cytotoxic activity; Antibacterial activity; Yucatec Maya; Traditional medicine

1. Introduction

Based on an ethnobotanical field study with the Yucatec Maya and an evaluation of their medicinal plants, the roots of *Crossopetalum gaumeri* (Loes.) Lundell were chosen for a detailed phytochemical study (Ankli et al., 1999; Ankli et al., submitted). A piece of root is chewed and the pulverized root is mixed with water and put on the wound of the person who is bitten by a snake in the form of a plaster. Furthermore, the decoction is used orally for diarrhoea. Crossopetalum is a genus in the family Celastraceae with 36 species in tropical America (Mabberly, 1987). This family is of great interest for phytochemical investigation especially due to the discovery of the antitumor effect of maytansinoides and other novel structure types isolated from Celastraceae (Brüning and Wagner, 1978). However, the genus Crossopetalum is not well investigated. From *C. tonduzii*, sesquiterpenes (Tincusi et al., 1998) and from *C. uragoga*, by Huastec Maya used as an anti-diarrheal medicine, triterpenes have been isolated (Domínguez et al., 1984). This report describes the isolation, structure elucidation, as well as cytotoxic and antibacterial activity of five cardiac glycosides (**2**–**6**), four terpenoids (**7**–**11**), and one catechin derivative (**1**) from the methanol and dichloromethane extracts of the roots of *C. gaumeri*.

2. Results and discussion

The methanol extract was fractionated using cytotoxicity against a KB cell line as a lead. This led to the isolation of the well known ourateacatechin (**1**) (Drewes and Mashimbye, 1993) and five cardenolide glycosides (**2**–**6**). All cardenolides were isolated as white amorphous powder and showed greenish to blue

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spots on TLC after spraying with vanillin–H_2SO_4 as well as an intense fluorescence under UV 366 nm.

The ^{1}H NMR spectrum of compound **2** showed characteristic signals of a butenolactone ring at δ 5.03, 4.92 (each *dd*, $J = 18.3$, 1.5 Hz, H-21a and b) and 5.92 (*s*, H-22), as well as a singlet proton at δ 9.41 indicating a cardenolide with an aldehyde function. A doublet at δ 4.67 ($J = 8.2$ Hz, H-1′), four additional protons between 3.99 and 3.46 ppm and a signal at δ 1.21 (*d*, $J = 6.7$ Hz, H_3-6′) pointed to the presence of a β-linked deoxyhexose (Table 1). ^{13}C NMR experiments, including DEPT 135, sorted 29 carbons into 2 methyl, 9 methylene, 13 methine and 5 quaternary carbons which is consistent with the molecular formula $C_{29}H_{42}O_{10}$ (Table 2). FAB-MS spectrum showed pseudomolecular peaks at *m/z* 551 $[M+H]^+$ and at *m/z* 573 $[M+Na]^+$. Fragments at *m/z* 404 and 146 confirmed the sugar to be a deoxyhexose (Ferth and Kopp, 1992; Habermeier, 1980). In accordance with the proposed molecular formula, positive HRESI-MS revealed a pseudomolecular ion at 551.2849 $[M+H]^+$. Based on COSY, HSQC, HMBC and ROESY experiments and in comparison to literature data, the aglycon was identified as securigenin (Kawaguchi et al., 1993; Ferth et al., 1992). Structure elucidation of the sugar moiety was performed on the basis of coupling constant analysis and of ROESY and COSY experiments. H-2′ resonated at 3.60 ppm as double doublet indicating an axial–axial relationship to H-1′ ($J = 8.2$ Hz) and an axial–equatorial relationship to H-3′ ($J =$ 3.5 Hz). Due to the small coupling constants of H-4′ (*dd*, $J = 3.5$ and 1.0 Hz) and a strong NOE between H-1′ and H-5′, H-4′ is in equatorial and H-5′ in axial position. Based on these data, the sugar moiety was identified as β-6-deoxygulose, which was confirmed by TLC after hydrolysis of **2** and comparison to authentic β-6-deoxygulose. Consequently, compound **2** was identified as the new securigenin-3β-*O*-β-6-deoxyguloside (**2**). COSY, HMBC and HSQC experiments showed unambiguously that C-2′ resonates at δ 68.1 and C-4′ at δ 72.3 (Table 2). Therefore, the ^{13}C chemical shift assignment for β-6-deoxygulose in literature must be corrected (Table 2).

The ^{13}C NMR spectrum of compound **3** showed

Table 1
^{1}H NMR data of compounds **2**–**6** (CD_3OD, δ ppm, *J* in Hz, 500 MHz; compound **6**, 600 MHz)[a]

H	2	3	4	5	6
1	2.15[b], 1.82[b]	2.47 *m*, 2.24 *m*	2.25[b], 1.84[b]	2.30 *br d* (13.4), 1.50[b]	2.18[b],1.82[b]
2	1.81[b]	1.88[b]	1.82[b], 1.74[b]	1.82[b],1.66[b]	1.80[b]
3	4.06 *br s*	4.21 *br s*	4.05 *br s*	4.01 *br s*	4.06 *m*
4	2.15[b], 1.73[b]	2.09[b], 1.73[b]	1.81[b], 1.60[b]	1.83[b], 1.54[b]	1.78[b], 1.70[b]
5	2.37 *m*	–	2.11 *m*	1.79[b]	2.36 *m*
6	1.76[b], 1.46[b]	1.88[b], 1.66[b]	1.78[b], 1.27[b]	1.86[b], 1.27[b]	1.46[b]
7	1.82[b]	2.07[b], 1.29[b]	1.83[b], 1.33[b]	1.80[b]	1.82[b], 1.26[b]
8	1.82[b]	2.01[b]	1.72[b]	1.66[b]	1.84[b]
9	1.82[b]	1.74[b]	1.89[b]	1.80[b]	1.82[b]
11	4.42 *m*	3.95[b]	3.82 *td* (10.5, 4.3)	3.72 *td* (10.4, 4.3)	4.42 *m*
12	1.75[b], 1.50[b]	1.68[b], 1.50 *dd* (13.7, 10.7)	1.69 *m*, 1.56 *br t* (12.1)	1.68[b], 1.56[b]	1.76[b], 1.53 *br t* (11.0)
15	2.18[b], 1.69[b]	2.16[b], 1.69[b]	2.25[b], 1.78[b]	2.21[b], 1.75[b]	2.18[b], 1.70[b]
16	2.18[b], 1.91[b]	2.15[b], 1.90[b]	2.22[b], 1.91[b]	2.19[b], 1.90[b]	2.17[b], 1.90[b]
17	2.93 *t* (7.1)	2.93 *t* (7.1)	2.93 *m*	2.91 *m*	2.93 *t* (6.9)
18	0.98 *s*	0.90 *s*	0.93 *s*	0.90 *s*	0.98 *s*
19	9.41 *s*	9.97 *s*	3.86 *d* (10.8), 3.71 *d* (10.8)	1.07 *s*	9.42 *s*
21	5.03 *dd* (18.3, 1.5)	5.03 *dd* (18.4, 1.5)	5.03 *dd* (18.2, 1.5)	5.02 *d* (18.3)	5.02 *dd* (18.5, 1.5)
	4.92 *dd* (18.3, 1.5)	4.92 *dd* (18.4, 1.5)	4.92 *dd* (18.2, 1.5)	4.91 *d* (18.3)	4.91 *dd* (18.5, 1.5)
22	5.92 *s*	5.92 *s*	5.94 *d* (1.0)	5.91 *s*	5.90 *s*
1′	4.67 *d* (8.2)	4.70 *d* (8.1)	4.67 *d* (8.2)	4.67 *d* (7.9)	4.70*d* (8.1)
2′	3.60 *dd* (8.2, 3.5)	3.59 *dd* (8.2, 3.3)	3.62 *dd* (8.1, 3.4)	3.35[b]	3.37 *dd* (8.1, 3.1)
3′	3.96[b]	3.96[b]	3.98[b]	4.33 *t* (2.9)	4.34 *t* (2.6)
4′	3.46 *dd* (3.8, 1.0)	3.46 *d* (3.4)	3.47 *d* (3.5)	3.27 *dd* (9.3,2.7)	3.28 *dd* (9.5, 2.6)
5′	3.99[b]	4.01 *q* (7.2, 1.0)	3.99[b]	3.85[b]	3.84[b]
6′	1.21 *d* (6.7)	1.22 *dd* (6.6)	1.23 *d* (6.7)	1.28 *d* (6.1)	1.29 *d* (6.2)
1″				4.73 *d* (7.9)	4.74 *d* (7.7)
2″				3.34[b]	3.35[b]
3″				4.05 *t* (2.9)	4.05 *m*
4″				3.54 *dd* (9.0, 2.7)	3.53 *dd* (8.8,2.8)
5″				3.69[b]	3.70[b]
6″				3.82[b], 3.70[b]	3.82[b], 3.70[b]

[a] Assignments are confirmed by COSY, HSQC and HMBC.
[b] Multiplicities are unclear due to overlapping.

close similarities to compound **2** with the exception of the signal of C-5 which resonated as quaternary carbon at δ 74.0 and downfield shifts of C-4 and C-6 pointing to an additional substitution with a hydroxyl group. In accordance, the positive FAB-MS spectrum exhibited a $[M+H]^+$ peak at *m/z* 567 and a fragment at 421 $[M+H\text{-deoxyhexosyl}]^+$. After extensive 1D and 2D NMR analysis, compound **3** was identified as sarmentosigenin-3β-*O*-β-6-deoxyguloside. It was described for the first time as canescein from the genus Erysimum (Maslennikova et al., 1967; Makarevich and Kovalev, 1968). The molecular formula of compound **4** was established as $C_{29}H_{44}O_{10}$, obtained from the ^{13}C NMR spectrum and the positive FAB-MS, showing a pseudomolecular peak at *m/z* 553 $[M+H]^+$. In contrast to securigenin-3β-*O*-β-6-deoxyguloside (**2**), in the ^1H NMR spectrum of **4**, the aldehyde proton is replaced by two protons resonating at δ 3.86 and 3.71 (each *d*, $J = 10.8$) pointing to a hydroxymethyl group.

Table 2
^{13}C NMR data of compounds **2**–**7**,**11** (CD_3OD, 75.5 MHz, δ ppm)

C[a]	2	3	4	5	6	C[a]	7	11
1	23.5 *t*	19.7 *t*	27.5 *t*	33.8 *t*	24.9 *t*	1	34.6 *t*	109.0 *d*
2	21.5 *t*	25.5 *t*	28.0 *t*	28.3 *t*	26.9 *t*	2	26.0 *t*	151.7 *s*
3	72.8 *d*	73.6 *d*	74.4 *d*	75.9 *d*	74.4 *d*	3	163.1 *s*	143.5 *s*
4	29.1 *t*	34.0 *t*	31.4 *t*	31.6 *t*	30.4 *t*	4	119.0 *s*	127.9 *s*
5	30.6 *d*	74.0 *s*	32.0 *d*	39.1 *d*	32.0 *d*	5	46.4 *d*	119.9 *s*
6	28.8 *t*	37.1 *t*	27.7 *t*	28.1 *t*	30.1 *t*	6	21.1 *t*	182.8 *s*
7	25.5 *t*	23.9 *t*	22.2 *t*	22.7 *t*	22.9 *t*	7	24.6 *t*	147.4 *s*
8	41.4 *d*	40.6 *d*	41.7 *d*	41.9 *d*	42.8 *d*	8	124.1 *s*	139.1 *s*
9	41.2 *d*	44.5 *d*	42.7 *d*	43.0 *d*	42.6 *d*	9	148.3 *s*	40.7 *s*
10	52.1 *s*	55.1 *s*	41.1 *s*	37.5 *s*	53.5 *s*	10	36.8 *s*	152.8 *s*
11	66.3 *d*	66.9 *d*	69.0 *d*	68.9 *d*	67.7 *d*	11	115.7 *d*	34.2 *t*
12	48.8 *t*	48.5 *t*	50.6 *t*	50.5 *t*	50.2 *t*	12	123.5 *d*	30.5 *t*
13	49.6 *s*	49.5 *s*	51.1 *s*	51.0 *s*	51.0 *s*	13	129.4 *s*	40.0 *s*
14	83.9 *s*	83.8 *s*	85.8 *s*	85.6 *s*	85.3 *s*	14	154.6 *s*	47.2 *s*
15	31.7 *t*	31.5 *t*	33.5 *t*	33.6 *t*	33.1 *t*	15	75.7 *s*	29.6 *t*
16	26.4 *t*	26.4 *t*	27.9 *t*	27.9 *t*	27.8 *t*	16	30.6 *q*	37.8 *t*
17	50.2 *d*	49.9 *d*	51.8 *d*	51.8 *d*	51.6 *d*	17	30.7 *q*	31.1 *s*
18	15.9 *q*	16.0 *q*	17.6 *q*	17.5 *q*	17.3 *q*	18	–	45.4 *d*
19	207.1 *d*	209.6 *d*	66.7 *t*	24.3 *q*	208.5 *d*	19	18.5 *q*	31.9 *t*
20	175.7 *s*	175.7 *s*	177.1 *s*	177.1 *s*	177.1 *s*	20	22.9 *q*	41.7 *s*
21	73.9 *t*	73.9 *t*	75.3 *t*	75.3 *t*	75.3 *t*	21		30.9 *t*
22	116.5 *d*	116.6 *d*	118.0 *d*	118.0 *d*	118.0 *d*	22		36.2 *t*
23	176.2 *s*	176.0 *s*	177.7 *s*	177.7 *s*	177.6 *s*	23		14.0 *q*
1′	99.1 *d*	98.5 *d*	100.0 *d*	99.9 *d*	99.8 *d*	24		-
2′	68.1 *d*	68.1 *d*	69.5 *d*	72.0 *d*	72.0 *d*	25		41.6 *q*
3′	72.2 *d*	72.1 *d*	73.5 *d*	72.4 *d*	72.4 *d*	26		19.6 *q*
4′	72.3 *d*	72.2 *d*	73.7 *d*	84.1 *d*	84.0 *d*	27		20.6 *q*
5′	68.6 *d*	68.8 *d*	69.9 *d*	69.4 *d*	69.5 *d*	28		32.1 *q*
6′	15.0 *q*	14.9 *q*	16.4 *q*	18.2 *q*	18.2 *q*	29		180.7 *s*
1″				103.5 *d*	103.5 *d*	30		32.9 *q*
2″				72.3 *d*	72.3 *d*	31		52.2 *q*
3″				73.1 *d*	73.1 *d*			
4″				68.4 *d*	68.4 *d*			
5″				75.2 *d*	75.2 *d*			
6″				62.6 *t*	62.6 *t*			

[a] Multiplicities determined by DEPT sequences.

As a result of MS and NMR analysis, the genin was identified as 19-hydroxysarmentogenin (Kopp and Kubelka, 1982). The sugar rest was identified as β-6-deoxygulose as described for compound **2**. Thus, compound **4** is the hitherto unknown 19-hydroxy-sarmentogenin-3β-*O*-β-6-deoxyguloside.

The ^1H and ^{13}C NMR of compound 5 indicated the presence of a cardenolide with two sugar moieties and a C-19 methyl group (Tables 1 and 2). The DEPT experiments sorted 35 carbons into 3 methyl, 10 methylene, 17 methine and 5 quaternary carbons. The positive ESI-MS spectrum gave a molecular ion at *m/z* 698. Therefore, the molecular formula of **5** was determined as $C_{35}H_{54}O_{14}$ and the aglycon was identified as sarmentogenin (Hanada et al., 1992). The identity and connectivities of the sugar moiety were deduced from a combination of 1D and 2D NMR experiments (^1H, ^1H COSY, HSQC, HMBC and ROESY) and confirmed by TLC hydrolysis and comparison with authentic compounds. Therefore, **5** was established as the new sarmentogenin-3β-*O*-[α-allosyl-(1 → 4)-β-6-deoxyalloside].

The aglycon of compound **6** showed nearly the same chemical shifts and correlations in the 1D and 2D NMR spectra as compound **2** and was therefore identified as securigenin. The chemical shifts and connectivities of the sugar moieties were identical with those of compound **5**. Therefore, **6** was identified as the new securigenin-3β-*O*-[α-allosyl-(1 → 4)-β-6-deoxyalloside].

All isolated cardiac glycosides (**2**–**6**) showed high cytotoxic activity against the KB cell line (Table 3). It is worthy of note that the cytotoxicity is not correlated with the oxidation status of the C-19 methyl group. Cytotoxicities of compounds **2** and **4** (IC_{50} 0.164 μmol **2** versus 0.199 μmol **4**) as well as **5** and **6** (IC_{50} 0.075 μmol **5** versus 0.104 μmol **6**) were in the same range. Comparison between compounds with identical genin and different sugar chains revealed a significant difference (IC_{50} 0.164 μmol **2** and IC_{50} 0.104 μmol **6**). Intro-

Table 3
Cytotoxicity of compounds **1**–**11** against a KB cell line (IC_{50} in μmol)

Compound	IC_{50}	Standard error	*n*
1	5.938	±0.1	4
2	0.164	±0.015	4
3	0.074	±0.009	4
4	0.199	±0.008	6
5	0.075	±0.004	8
6	0.104	±0.005	6
7	> 66	–	4
8	> 45	–	4
9	0.603	±0.01	4
10	1.6	±0.14	4
11	9.476	±0.35	4
Podophyllotoxin	0.014	–	4

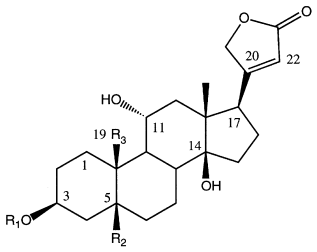
duction of a hydroxyl group at C-5 doubled the cytotoxicity (IC_{50} 0.164 μmol **2** versus 0.074 μmol **3**).

Bioactivity-guided fractionation of the dichloromethane extract, using antibiotic activity against *Bacillus cereus*, *Staphylococcus epidermidis* and *Micrococcus luteus* led to the isolation of the new diterpene, 3,15-dihydroxy-18-norabieta-3,8,11,13-tetraene (**7**), the three known triterpenes, friedelane-3-on-29-ol (**8**), pristimerin (**9**) (Gunatilaka et al., 1989), and celastrol (**10**) (Kutney et al., 1981; Patra and Chaudhuri, 1987), and the new triterpene 2,3,7-trihydroxy-6-oxo-1,3,5(10),7-tetraene-24-nor-friedelane-29-oic acid methylester (**11**). Compound **7** was isolated as a pale yellow amorphous powder. The ^{13}C NMR spectrum showed signals of 19 carbons, which could be assigned with DEPT experiments into 4 methyl, 4 methylene, 3 methine and 8 quaternary carbons. The positive EI-MS, showing a molecular peak at m/z 302 in combination with the ^{13}C NMR spectra allowed the establishment of the molecular formula $C_{19}H_{26}O_3$. The ^1H, ^1H TOCSY and COSY spectra revealed three spin systems. Spin system A with two coupling protons at δ 6.79 and 6.91 (each *d*, $J = 8.3$, H-11 and H-12) indicated the presence of a 1,2,3,4-tetra-substituted aromatic ring. Spin systems B (H-5/H-6/H-7) and C (H-1/H-2) belong to a CH–CH_2–CH_2 and a CH_2–CH_2 moiety, respectively. The linkage between the spin systems was established by a HMBC experiment and led to a norabietan skeleton with a C-3/C-4 double bond (Takaishi et al., 1997a). HSQC, HMBC and ROESY were utilized to clarify the ^1H and ^{13}C NMR assignments and the stereochemistry. Taken together, the data established the structure 3,15-dihydroxy-18-norabieta-3,8,11,13-tetraene (**7**). ^{13}C NMR data clearly revealed that we isolated only the enol form **7** whereas the tautomeric ketone is completely absent.

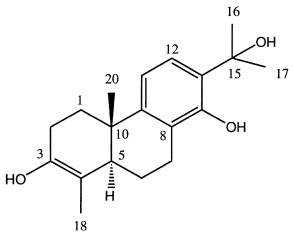
The molecular formula of compound **11**, a yellow amorphous powder, was established as $C_{30}H_{40}O_6$, obtained from the ^{13}C NMR spectrum and from positive EI-MS, showing the molecular peak at m/z 496 $[M]^+$. The ^{13}C NMR and the DEPT revealed the presence of 7 methyl, 7 methylene, 2 methine and 14 quaternary carbons. The UV spectrum contained absorption maxima at 248 and 321 nm due to a phenolic ring system. The ^1H and ^{13}C NMR data showed chemical shifts similar to those of the C, D and E ring of pristimerin (**9**) (Gunatilaka et al., 1989). For rings A and B, long range correlations of the proton H-1 with the carbons C-2, C-3, C-4, C-5, C-6, C-10 and the protons H_3-23 with C-1, C-2, C-3, C-4, C-5, C-6 and C-10 were observed. These data and comparison with literature data of regeol C (Takaishi et al., 1997b) led to the identification of its methylester as 2,3,7-trihydroxy-6-oxo-1,3,5(10),7-tetraene-24-nor-friedelane-29-oic acid methylester (**11**).

Confirming earlier published data, the triterpenoids **9** and **10** showed high antibacterial activity particularly against *S. epidermidis* as well as a remarkable cytotoxicity against KB cells (Kutney et al., 1981; González et al., 1998). Both new terpenoids **7** and **11** lack cytotoxicity and the antibacterial activity is moderate to low (Tables 3 and 4).

The antibacterial activity of pristimerin (**9**) seems to be an explanation of the use of *C. gaumeri* as an antidiarrheal medicine. Due to its cytotoxic potential *C. gaumeri* and preparations thereof should be used with great caution.



	R_1	R_2	R_3
2	β-6-deoxygulosyl	H	CHO
3	β-6-deoxygulosyl	OH	CHO
4	β-6-deoxygulosyl	H	CH_2OH
5	α-allosyl-(1→4)-β-6-deoxyallosyl	H	CH_3
6	α-allosyl-(1→4)-β-6-deoxyallosyl	H	CHO



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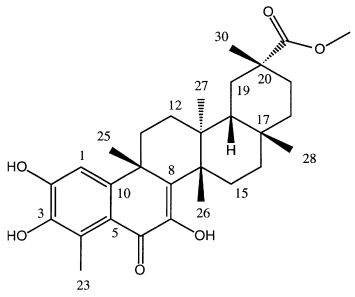

11

3. Experimental

3.1. General experimental procedures

Optical rotations were measured in MeOH or $CHCl_3$ on a Perkin-Elmer model 241 polarimeter. UV spectra were obtained on a Kontron–Uvikon 930 spectrophotometer, using MeOH as a solvent. EI-MS were measured on a Hitachi–Perkin-Elmer RMUGM mass spectrometer at 70 eV. FAB-MS were obtained in the positive mode on a ZAB 2-SEQ spectrometer, using 3-nitrobenzylalcohol as matrix. ESI-MS (positive mode) were measured on a TSQ 7000 mass spectrometer. Applying HRESI-MS (positive mode) to the cardiac glycosides only compound **2** expressed a detectable $[M+H]^+$ pseudomolecular ion. ^1H and ^{13}C NMR

Table 4
Antibacterial activities of compounds **7**–**11** (MIC in μmol)

Compound	Minimum inhibition concentration (MIC) in broth		
	B. cereus	*S. epidermidis*	*M. luteus*
7	423.84	–	–
8	–	–	–
9	8.62	0.54	8.62
10	4.44	1.11	4.44
11	129.03	129.03	32.26
Chloramphenicol	6.19	12.38	6.19

spectra were recorded using Bruker AMX-300, DRX-500 and DRX-600 spectrometers. The spectra were measured in CD_3OD or $CDCl_3$ and the residual CH_3OH and $CHCl_3$ resonances were used as internal references. For VLC, silica gel ($60F_{254}$, 40–60 μm, Merck) and RP-18 material (40–63 μm, CU Chemie Uetikon AG) was used. MPLC was carried out using a Büchi chromatography pump B-688 and a 3.5×80 cm Büchi column packed with silica gel ($60HF_{254}$, 15 μm, Merck). HPLC separations were performed with a Merck–Hitachi L-6200 intelligent pump connected to a Merck–Hitachi L-4000 UV detector or a Waters model 590 pump connected to a Pharmacia Biotech Uvicord SII detector. HPLC columns were from Knauer (Spherisorb S5 ODSII, 250×16 mm, ECT Chemie AG, 5 μm for the MeOH extract and Spherisorb ODSII, 250–20 mm, Waters, 10 μm for the CH_2Cl_2 extract).

3.2. Plant material

C. gaumeri was collected from the villages and surroundings of Chikindzonot, Ekpedz and Xcocmil, Yucatan, Mexico (1994–1995). Authenticated voucher specimens were deposited at the Herbarium of the Centro de Investigación Científica de Yucatán (CICY) in Mérida, the National Herbarium of Mexico (MEXU), the Instituto Nacional Indigenista (INI) in Valladolid, Yucatan, the ETH Zurich (ZT) and the Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, London, UK.

3.3. Extraction and isolation

Air-dried and powdered roots of *C. gaumeri* (2.26 kg) were successively extracted with CH_2Cl_2, MeOH, 70% aqueous MeOH and H_2O. Extraction with CH_2Cl_2 yielded 42 g extract and from the methanol extraction 95 g were obtained. An aliquot of this extract (42 g) was partitioned between $CHCl_3$ and 60% aqueous MeOH (1:1). The polar fraction was further partitioned between *n*-butanol and H_2O (1:1). The butanol fraction was combined with the $CHCl_3$ one and applied to VLC, as two separate portions, of 10 g each. Elution with $CHCl_3$ containing increasing amounts of methanol yielded 18 fractions. Fraction 9 ($CHCl_3$–MeOH, 9:1) yielded compound **1** (130 mg).

Fractions 13–15 (6.9 g, $CHCl_3$–MeOH, 9:1 to 6:4) were combined and subjected to an open column chromatography using silica gel 60 (35–70 μm) as stationary phase and increasing amounts of aqueous MeOH (MeOH–H_2O, 99:1) in $CHCl_3$ as eluent (100–45% $CHCl_3$) to give 17 subfractions. The subfractions 2 (83.5:16.5), 5 (78:22), 7 (70:30), 11 (67:33) and 14 (50:50) were purified by HPLC on RP-18 using ACN–H_2O (20:80) as mobile phase for the subfractions 2, 5 and 7 and ACN–H_2O (15:85) for the subfractions 11 and 14 yielding the compounds **2**–**6**.

An aliquot (33 g, 35%) of the CH_2Cl_2 extract was fractionated over silica gel (VLC), using *n*-hexane–EtOAc mixtures with increasing polarity as eluent to afford 22 frs. Fraction 8 (80:20) contained compound **9**. Frs 11 (30:70) and 12 (30:70) were combined and subjected to normal-phase MPLC employing *n*-hexane–EtOAc–MeOH (15:3:0.5 to 10:10:5) mixtures as mobile phase. Based on TLC control, the subfractions were combined to give 21 fractions. Fraction 3 (15:3:1) contained compound **9**, after purification of fraction 6 (12:8:1) based on MeOH-solubility, compound **8** was isolated. Fr 13 (10:10:3) was purified on a C18 Sep-Pak® Cartridge with 50% aqueous MeOH increasing the methanol proportion to give **7**. Fraction 8 (10:10:1) was separated by RP-VLC using increasing amount of MeOH in water as eluent to give 38 fractions. Of these, fractions 24 and 25 (MeOH–H_2O, 9:1) were combined and refractionated by a silica VLC using *n*-hexane and *n*-hexane–$CHCl_3$ mixtures with increasing polarity which afforded 19 fractions. Fractions 10–15 (20:80) were combined and purified by RP-HPLC (ACN–H_2O, 9:1) to yield **10** and **11**.

3.4. Detection of cardiac glycosides and hydrolysis of the cardiac glycosides on TLC plates

As a mobile phase for TLC analysis of the cardenolides, EtOAc–MeOH–H_2O (81:11:8) was used. For detection vanillin–H_2SO_4 was sprayed on the TLC plates (silica gel $60F_{254}$) and heated at 110°C for 5–10 min. The evaluation was carried out under vis and under UV light 366 nm. The TLC hydrolysis of the cardiac glycosides was realised following Kartnig and Wegschaider (1971) with some modifications. The tank was not saturated with 36% HCl and the TCL plate was exposed to HCl vapour for 5 min (100°C). The plate was then dried for 2 h in the air and 30 min on a heating plate (80°C). Development was carried out with $CHCl_3$–MeOH–H_2O (64:36:8) as mobile phase and it was sprayed with 0.5 g thymol in 95 ml EtOH and 5 ml H_2SO_4 (concentrate).

3.5. Cytotoxicity study using KB cell culture

The cytotoxicity of the compounds was determined using a KB cell line (ATCC CCL 17; human nasopharyngeal carcinoma). The test was carried out with some modifications according to the screening technique of Swanson and Pezzuto (1990) in 96-well plates (Falcon) with an inoculum of 2.5×10^4 cells/ml. Test solutions were made as stocks in 20% ethanol in water. Before testing, the solutions were diluted 20-fold and the final ethanol concentration was 1% (v/v) or less. The total assay volume was 150 μl. For quantification of cytotoxicity, 15 μl of an aqueous solution of methylthiazolyltetrazolium chloride (MTT, Fluka) with 5 mg/ml PBS was added (Mosmann, 1983). After incubation at 37°C for 4 h, the metabolically active cells produced an insoluble formazan dye. The medium was drawn off and the formazan dye was dissolved using 150 μl of 10% SDS (sodium dodecylsulfate) in water. After 24 h of incubation at room temperature, the optical density was measured at 540 nm using a microplate reader (MRX, Dynex Technologies). For determination of the IC_{50} values, the optical density was plotted against the log concentration. The test was performed at least in duplicates.

3.6. Antibacterial activity

Antibacterial activity against *B. cereus* (ATCC 10702), *S. epidermidis* (ATCC 12228), *M. luteus* (ATCC 9341) and *E. coli* (ATCC 25922) were assessed using the doubling dilution method (Liu et al., 1999).

3.7. Securigenin-3β-O-β-6-deoxyguloside (**2**)

White powder (11.1 mg); $[\alpha]_D^{24}$ −61.0° (MeOH, *c* 1.0); UV λ_{max} (MeOH): 217 nm; positive FAB-MS *m/z* 573 $[M+Na]^+$, 551 $[M+H]^+$, 443 [M + K-deoxyhexosyl]$^+$, 369 [M + H-desoxyhexosyl-$2H_2O$]$^+$; positive HRESI-MS 551.2849 $[M+H]^+$ (calculated 551.2856); ^{1}H NMR (500 MHz, CD_3OD) (Table 1); ^{13}C NMR (75.5 MHz, CD_3OD) (Table 2).

3.8. Sarmentosigenin-3β-O-β-6-deoxyguloside (**3**)

White powder (9.1 mg); $[\alpha]_D^{24}$ −26.0° (MeOH, *c* 2.3); UV λ_{max} (MeOH): 214 nm; positive FAB-MS *m/z* 567 $[M+H]^+$, 421 [M + H-deoxyhexosyl]$^+$; ^{1}H NMR (500 MHz, CD_3OD) (Table 1); ^{13}C NMR (75.5 MHz, CD_3OD) (Table 2).

3.9. 19-Hydroxy-sarmentogenin-3β-O-β-6-deoxyguloside (**4**)

White powder (23.4 mg); $[\alpha]_D^{24}$ −36.0° (MeOH, *c* 1.0); UV λ_{max} (MeOH): 214 nm; positive FAB-MS *m/z* 553 $[M+H]^+$; ^{1}H NMR (500 MHz, CD_3OD) (Table 1); ^{13}C NMR (75.5 MHz, CD_3OD) (Table 2).

3.10. Sarmentogenin-3β-O-[α-allosyl-(1 → 4)-β-6-deoxyalloside] (**5**)

White-brown powder (39 mg); $[\alpha]_D^{24}$ −5.2° (MeOH, *c* 2.3); UV λ_{max} (MeOH): 214 nm; positive ESI-MS *m/z* 721 $[M+Na]^+$, 698 $[M]^+$; positive FAB-MS *m/z* 573 [M + K-H-hexosyl]$^+$; ^{1}H NMR (500 MHz, CD_3OD) (Table 1); ^{13}C NMR (75.5 MHz, CD_3OD) (Table 2).

3.11. Securigenin-3β-O-[α-allosyl-(1 → 4)-β-6-deoxyalloside] (**6**)

White-yellow powder (2.4 mg); $[\alpha]_D^{24}$ −28.7° (MeOH, *c* 2.2); UV λ_{max} (MeOH): 214 nm; positive ESI-MS *m/z* 735 $[M+Na]^+$, 712 $[M]^+$; positive FAB-MS *m/z* 573 [M + Na + H-hexosyl]$^+$; ^{1}H NMR (600 MHz, CD_3OD) (Table 1); ^{13}C NMR (75.5 MHz, CD_3OD) (Table 2).

3.12. 3,15-Dihydroxy-18-norabieta-3,8,11,13-tetraene (**7**)

Yellow-brown powder (6.9 mg); $[\alpha]_D^{24}$ +23.5° (MeOH, *c* 2.9); UV λ_{max} (MeOH): 273 nm; positive EI-MS *m/z* 302 $[M]^+$, 301, 258, 216, 202, 188, 173, 149, 85, 83, 49; ^{1}H NMR (300 MHz, CD_3OD) δ: 1.04 (3H, *s*, H-20), 1.53 (1H, *m*, H-1b), 1.56 (3H, *s*, H-17), 1.58 (3H, *s*, H-16), 1.62 (1H, *m*, H-6b), 1.93 (3H, *brs*, H-18), 2.23 (1H, *m*, H-5), 2.29 (1H, *m*, H-6a), 2.36 (1H, *m*, H-1a), 2.48 (2H, *brd*, $J = 3.7$ Hz, H-2), 2.70 (1H, *dd*, $J = 9.8$, 18.9 Hz, H-7b), 2.90 (1H, *dd*, $J = 7.2$, 18.4 Hz, H-7a), 6.79 (1H, *d*, $J = 8.3$, H-11), 6.91 (1H, *d*, $J = 8.3$, H-12); ^{13}C NMR (75.5 MHz, CD_3OD) (Table 2).

3.13. 2,3,7-Trihydroxy-6-oxo-1,3,5(10),7-tetraene-24-nor-friedelane-29-oic acid methylester (**11**)

Yellow powder (5.3 mg); $[\alpha]_D^{24}$ − 45.5° (MeOH, *c* 3.03); UV λ_{max} (MeOH): 284, 321 nm; positive EI-MS *m/z* 496, 263, 248, 234, 203, 44; ^{1}H NMR (300 MHz, CD_3OD) δ: 0.69 (3H, *s*, H-27), 0.96 (1H, *m*, H-22b), 1.08 (3H, *s*, H-28), 1.15 (3H, *s*, H-30), 1.37 (3H, *s*, H-26), 1.43 (1H, *m*, H-21b), 1.45 (1H, *m*, H-16b), 1.49 (3H, *s*, H-25), 1.59 (1H, *d*, $J = 7.8$ Hz, H-18), 1.66 (1H, *dt*, $J = 4.1$, 14.1 Hz, H-12b), 1.71 (1H, *dd*, $J = 7.4$, 14.7 *Hz*, H-19b), 1.82 (1H, *m*, H-12a), 1.85 (1H, *m*, H-15b), 1.91 (1H, *m*, H-16a), 1.97 (1H, *dt*, $J = 4.2$, 14.2 Hz, H-11b), 2.12 (1H, *dt*, $J = 4.1$, 13.8 Hz, H-22a), 2.16 (1H, *m*, H-11a), 2.17 (1H, *m*, H-21a), 2.47 (1H, *brd*, $J = 15.5$ Hz, H-19a), 2.57 (3H, *s*, H-23), 2.88 (1H, *m*, H-15a), 3.55 (3H, *s*, H-31), 6.86 (1H, *s*, H-1); ^{13}C NMR (75.5 MHz, CD_3OD) (Table 2).

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