Phytochemistry 69 (2008) 2209–2213

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Nitrile glucosides and serotobenine from *Campylospermum glaucum* and *Ouratea turnarea*

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ARTICLE INFO

Article history:
Received 19 July 2007
Received in revised form 18 February 2008
Available online 10 June 2008

Keywords:
Campylospermum glaucum
Ouratea turnarea
Ouratea
Ochnaceae
Nitrile glucosides
Lanceolin C
Campyloside A
Campyloside B
Serotobenine
Flavonoids
Antimicrobial activity

ABSTRACT

Two new nitrile glucosides (1*S*,3*S*,4*S*,5*R*)-4-benzoyloxy-2-cyanomethylene-3,5-dihydroxycyclohexyl-1-*O*-β-glucopyranoside (campyloside A) and (1*S*,3*S*,4*S*,5*R*)-5-benzoyloxy-2-cyanomethylene-3-hydroxy-4-(2-pyrrolcarboxyloxy)cyclohexyl-1-*O*-β-glucopyranoside (campyloside B) were isolated from the stem roots of *Campylospermum glaucum*, whereas serotobenine was isolated from *Ouratea turnarea*. The structure elucidations were based on spectroscopic evidence.
The biological assays of compounds and crude extract of plant species showed good antimicrobial activity of crude extracts against Gram-positive cocci.

1. Introduction

Previous phytochemical analyses of the genus *Ouratea* have resulted in the isolation of flavonoids and diterpenoids (Moreira et al., 1994, 1999; Felicio et al., 1995, 2001, 2004; Velandia et al., 1998; Mbing et al., 2003; Pegnyemb et al., 2005). However, neither nitrile glucoside, nor serotobenine (Sato et al., 1985) has been reported from this genus even though a derivative of menisdaurin (Takahashi et al., 1978) has been isolated from *Ouratea reticulata* (Elo Manga et al., 2001). Nitrile glucosides have so far been reported only from the *Lophira* and *Ochna* genera of the Ochnaceae family to which *Ouratea* belongs (Murakami et al., 1993; Tih et al., 1994, 2003; Messanga et al., 1998a, 2002). In continuation of our phytochemical studies on *Ouratea* species or related species, the chemical constituents of the roots of *Campylospermum glaucum* (Tiegh) Farron, and *Ouratea turnarea* (Hook) Hutch & Dalz have been investigated. No previous chemical investigation has been reported on these species. We report in this paper the results of some bioassays, the isolation and structural elucidation of two new nitrile glucosides, campyloside A (**1**) and campyloside B (**2**).

2. Results and discussion

The roots of *C. glaucum*, and *O. turnarea* were extracted (separately) at the beginning with MeOH; the resulting gums were then subjected to a new extraction in a mixture of CH_2Cl_2/MeOH (1/1) to yield two different crude extracts, which were submitted to phytochemical analyses and antibacterial assays. The different resulting extracts were repeatedly chromatographed over silica gel and Sephadex LH-20 followed by recrystallisation. Final purification of *C. glaucum* extracts gave two nitrile glucosides in addition with lophirone A (Ghogomu et al., 1987), amentoflavone (Chari et al., 1977), whereas *O. turnarea* gave, apart from the flavonoids already cited, serotobenine together with lophirone C (Tih et al., 1989), isolophirone C (Pegnyemb et al., 2001) and calodenin B (Messanga et al., 1994).

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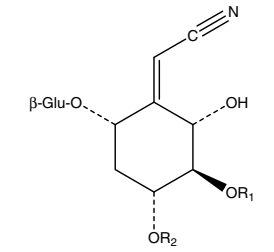
doi:10.1016/j.phytochem.2008.04.013



1- R_1 = Bz, R_2 = H

2- R_1 = , R_2 = Bz

2.1. Characterisation of campyloside A, *1*

Compound **1** was isolated as a white solid. Its mass spectrum exhibited a diagnostic ion at *m*/*z*: 452.162 $[M+H]^+$, suggesting the presence of a nitrogen atom in the molecule and a molecular formula of $C_{21}H_{25}O_{10}N$ (calcd. for $C_{21}H_{26}O_{10}N$: 452.156). This formula was further strengthened by a signal at δ_C 117.7 (C-8) (Table 1) in the ^{13}C NMR spectrum, indicating the presence of a nitrile group in the molecule.

The presence of a β-glucopyranosyl moiety was indicated by the signal of the anomeric proton at δ_H 4.38 (*d*, *J* = 7.8 Hz, H-1″) and in agreement with carbon signals at δ_C 102.2 (C-1″), 78.3 (C-5″), 77.9 (C-3″), 74.6 (C-2″), 71.2 (C-4″) and 62.3 (C-6″). The presence of a mono-substituted aromatic ring was suggested by ^1H NMR signals at δ_H 8.04 (*m*, *J* = 8.2, 1.6 and 1.5 Hz, H-2′, H-6′), 7.53 (*m*, *J* = 8.2, 8.0 and 1.1 Hz, H-3′, H-5′) and 7.66 (*m*, *J* = 8.0, 8.0 and 1.1 Hz, H-4′). In the ^{13}C NMR spectrum, four signals at δ_C 79.2 (C-4), 75.3 (C-1), 69.2 (C-3), 66.9 (C-5) were assignable to carbons linked to an oxygen atom, one at δ_C 35.3 (C-6) to a methylene group, and another one at δ_C 166.5 to a carbon of a conjugated ester. This conjugation was confirmed by the benzoyl group with aromatic carbon signals at δ_C 134.6 (C-4′), 131.3 (C-1′), 130.8 (C-2′, C-6′), 129.9 (C-3′, C-5′). The remaining carbons throughout the ^1H NMR data indicated the presence of a trisubstituted double bond involving the methine at δ_H 5.78 (*d*, *J* = 1.5 Hz, H-7) and δ_C 96.3 (C-7) linked to the quaternary sp^2 carbon at δ_C 165.0 (C-2).

Further elements from the HMBC spectrum provided three-bond correlations between anomeric proton at δ_H 4.38 and the carbon at δ_C 75.3 (C-1), the proton at δ_H 4.79 (*J* = 8.5 and 8.3 Hz, H-4) and the carbonyl of the benzoyl group (Fig. 1). Moreover, other correlations mentioned the presence of a cyclohexyl unit which is part of the aglycone moiety. The NOESY spectrum revealed the main interactions between the various protons, confirming the ordering of carbon and hydrogen atoms of the cyclohexyl unit and the glycoside bond. This glycoside bond involved the anomeric proton at δ_H 4.38 and the proton at δ_H 4.87 (*J* = 10.7 and 4.6 Hz, H-1). From these spectral data, it is unambiguously established that **1** appears as an isomer of lanceolins A, B (Tih et al., 1994) and principally of lanceolin C (Messanga et al., 1998a) previously reported. The coupling constants of protons of the cyclohexyl unit indicated, however, clearly the *trans*-relationship between H-5 (δ_H 3.08; *J* = 8.8, 8.3 and 3.8 Hz) and H-4 which also evolved a *trans*-relationship with H-3 (δ_H 4.74; *J* = 8.5 and 1.5 Hz), confirmed by an absence of correlation in the NOESY spectrum between H-3 and H-4 or H-4 and H-5. These additional data emphasize the difference on stereochemistry among **1** and lanceolin C, while their structure corresponds to 4-benzoyloxy-2-cyanomethylene-3,5-dihydroxycyclohexyl-1-*O*-β-glucopyranoside (Fig. 2).

Although the structure of lanceolin C has been reported previously from *Lophira alata* (Messanga et al., 1998a) with a complete set of coupling constants and chemical shifts, its physical data are not quiet related to those of compound **1** (Table 1), namely campyloside A.

Table 1
NMR spectral data for campyloside A (**1**) (DMSO-d_6); δ (ppm); *J* (Hz)

Number	δ_C	δ_H	*m*	*J* (→H atom)[a]
1	75.3	4.87	*dd*	10.7 (6b); 4.6 (6a)
2	165.0	–	–	
3	69.2	4.74	*dd*	8.5 (4); 1.5 (7)
4	79.2	4.79	*dd*	8.5 (3); 8.3 (5)
5	66.9	3.08	*m*	8.8 (6b); 8.3 (4); 5.3 (6a)
6a[b]	35.3	2.23	*ddd*	14.6 (6b); 5.3 (5);4.6 (1)
6b[b]		1.94	*ddd*	14.6 (6a); 10.7 (1); 8.8 (5)
7	96.3	5.77	*d*	1.5 (3)
8	117.7	–	–	
1′	131.3	–	–	
2′/6′	130.8	8.04	*m*	~8.2 (3′/5′); ~1.6 (6′/2′); ~1.5 (4′)**
3′/5′	129.9	7.53	*m*	~8.2 (2′/6′); ~8.0 (4′); 1.1 (5′/3′)
4′	134.6	7.66	*m*	~8.0 (3′/5′); ~1.5 (2′/6′)
7′	166.5	–	–	
1″	102.2	4.38	*d*	7.8 (2″)
2″	74.6	3.03	*ddd*	8.5 (3″); 7.8 (1″); 3.3 (4″)
3″	77.9	3.18	*ddd*	8.5 (2″); 8.5 (4″); 4.5 (5″)
4″	71.2	3.10	*m*	
5″	78.3	3.12	*m*	
6″a[c]	62.3	3.64	*dd*	11.2 (6″b)
6″b[c]		3.47	*m*	11.2 (6″a)

[a] Proton involved in the coupling.
[b] Protons on C-6.
[c] Protons on C-6″.

2.2. Characterisation of campyloside B, *2*

The ESI mass spectrum of **2** exhibited a diagnostic ion $[M+H]^+$ at *m*/*z* 545.180, suggesting the presence of two nitrogen atoms and a

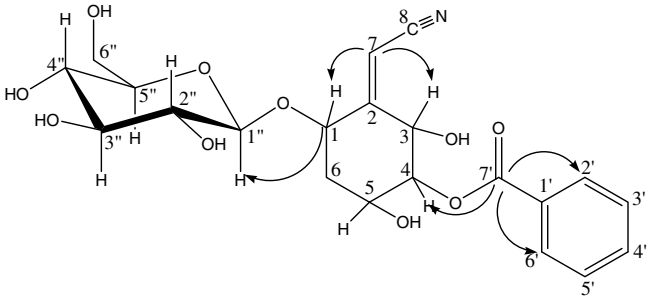


Fig. 1. HMBC correlations of campyloside A (**1**).

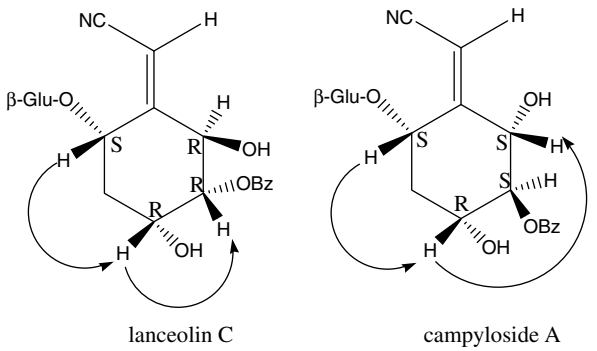


Fig. 2. Some characteristic NOESY correlations of lanceolin C and campyloside A (**1**).

molecular formula of $C_{26}H_{28}O_{11}N_2$ (calcd. 545.177). Its ^{13}C NMR spectrum showed the presence of 26 carbon atoms, several of which showed characteristic chemical shifts similar to those of compound **1**.

The signal at δ_C 104.4 (Table 2) was assigned to the anomeric carbon of a glucoside (C-1‴), while a nitrile group was noticeable at δ_C 117.1 (C-8). Additional peaks at δ_C 78.1; 77.9; 74.8; 71.8 and 63.2 support the idea that **2** contained a β-glucopyranose moiety. The peaks of aromatic carbons appeared at δ_C 133.9 (C-4″), 131.4 (C-1″), 130.7 (C-2″, C-6″), 129.2 (C-3″, C-5″); the peaks at δ_C 76.8 (C-1), 76.2 (C-4), 70.3 (C-5), 68.9 (C-3) are assignable to those of carbons bearing oxygen when the peak of the methylene group was observed at δ_C 33.7 (C-6). The conjugated double bond was recognizable at δ_C 165.2 (C-2) while the carbon signals at δ_C 160.5 (C-6′) and 166.1 (C-7″) designated conjugated esters. Carbon signals at δ_C 124.6 (C-5′), 122.9 (C-2′), 116.4 (C-3′) and 110.4 (C-4′) suggested the presence of a pyrrole moiety, thus confirming the occurrence of a second nitrogen in the molecule, in addition to the first one involved in the nitrile group.

The ^1H NMR spectrum confirms the presence of a glucosyl group due to the doublet at δ_H 4.44 (J = 7.8 Hz, H-1‴) and a series of characteristic protons from δ_H 3.22–3.88. The signal at δ_H 10.93 was assigned to a proton linked to a nitrogen atom when the other one at δ_H 5.93 (J = 1.9 Hz, H-7) traduced an olefinic proton. The NOESY spectrum indicated correlations between the proton at δ_H 5.07 (J = 10.6 and 3.5 Hz, H-1) and the anomeric proton at δ_H 4.44 (Fig. 4). Moreover, three-bond correlations were found in the HMBC spectrum (Fig. 3) between the anomeric proton at δ_H 4.44 and the carbon at δ_C 76.8 (C-1), the proton at δ_H 5.71 (J = 9.1, 8.6 and 3.1 Hz, H-5) and the carbonyl of the benzoyl group at δ_C 166.1 (C-7″); additional correlations occurred between the carbonyl group at δ_C 160.5 (C-6′) and the proton at δ_H 5.04 (J = 10.2 and 8.6 Hz, H-4) in one side, and with the proton at δ_H 6.68 (m, H-3′) in another side. Taken collectively, the above data support the idea for a similar skeleton for both campylosides A (**1**) and B (**2**) in the absence of the pyrrole group; however, according to all spectral data including HMBC and NOESY, there is a change in the location of the benzoyl group which confers to **2** a structure nearer to lanceolin B (Tih et al., 1994) than to campyloside A. The main differences concern the position of the benzoyl group in comparison with the pyrrole one, and the stereochemistry of the asymmetric carbons of the cyclohexyl moiety. The location of the benzoyl unit was emphasized by the NOESY spectrum where correlations were remarkable between the aromatic protons at δ_H 8.12 (H-2″/6″) and the protons H-2‴ at δ_H 3.22 of the glucopyranosyl moiety and H-1. Taking also in account the coupling constants of H-4 and H-5 (Table 2), there is no doubt about the *trans*-relationship between these two protons and consequently between the benzoyl and pyrrole groups (Fig. 4).

The structure of **2** was therefore determined as (1*S*,3*S*,4*S*,5*R*)-5-benzoyloxy-2-cyanomethylene-3-hydroxy-4-(2-pyrrolcarboxyloxy)-cyclohexyl-1-*O*-β-glucopyranoside, namely campyloside B.

Table 2
NMR spectral data for campyloside B (**2**) (DMSO-d_6); δ (ppm); J (Hz)

Number	δ_C	δ_H	m	J (→H atom)[a]
1	76.8	5.07	*dd*	10.6 (6b); 3.5 (6a)
2	165.2	–	–	
3	68.9	5.43	*dd*	10.2 (4); 1.9 (7)
4	76.2	5.04	*dd*	10.2 (3); 8.6 (5)
5	70.3	5.71	*m*	9.1 (6b); 8.6 (4); 3.1 (6a)
6a[b]	33.7	2.63	*ddd*	15.7 (6b); 3.5 (1); 3.1 (5)
6b[b]		2.28	*ddd*	15.7 (6a); 10.6 (1); 9.1 (5)
7	96.2	5.93	*d*	1.9 (3)
8	117.1	–	–	
1′	–	10.93	*s*	
2′	122.9	–	–	
3′	116.4	7.00	*m*	~7.1 (4′); ~2.6 (5′)
4′	110.4	6.11	*m*	~7.1 (3′); ~7.7 (5′)
5′	124.6	6.68	*m*	~7.7 (4′); ~2.6 (3′)
6′	160.5	–	–	
1″	131.4	–	–	
2″/6″	130.7	8.12	*m*	~8.0 (3″/5″); ~1.5 (4″); ~1.2 (6″/2″)
3″/5″	129.2	7.49	*m*	~8.0 (2″/6″); ~7.5 (4″); ~1.1 (5″/3″)
4″	133.9	7.62	*m*	~7.5 (3″/5″); ~1.5 (2″/6″)
7″	166.1	–	–	
1‴	104.4	4.44	*d*	7.8 (2‴)
2‴	74.8	3.22	*ddd*	8.4 (3‴); 7.8 (1‴); 2.4 (4‴)
3‴	78.1	3.42	*dd*	8.4 (2‴); 8.4 (4‴)
4‴	71.8	3.36	*dd*	8.4 (3‴); 8.4 (5‴)
5‴	77.9	3.31	*m*	8.4 (4‴)
6‴a[c]	63.2	3.88	*d*	11.4 (6‴b)
6‴b[c]		3.66	*m*	11.4 (6‴a)

[a] Proton involved in the coupling.
[b] Protons on C-6.
[c] Protons on C-6‴.

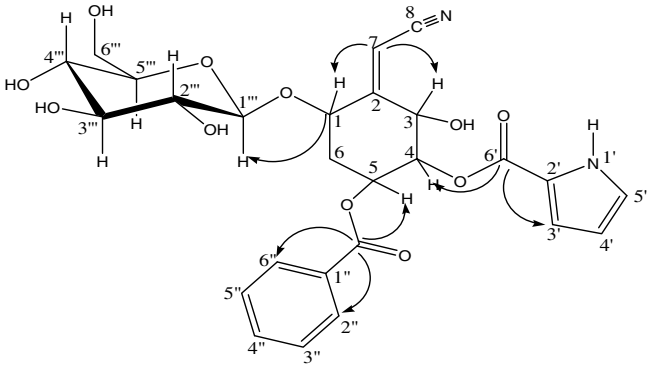


Fig. 3. HMBC correlations of campyloside B (**2**).

2.3. Chemotaxonomy

The present study afforded campylosides A and B, lophirone A and amentoflavone from *C. glaucum*; serotobenine, amentoflavone, lophirone C, isolophirone C and calodenin B from *O. turnarea*.

No nitrile glucoside or pyrrole alkaloïd has been reported so far from the *Ouratea* or *Campylospermum* genera. These genera can then be considered as a possible source of alkaloids. However, nitrile glucosides and biflavonoids are widespread in the Ochnaceae family (Murakami et al., 1993; Tih et al., 1994; Messanga et al., 1994, 1998a,b, 2002; Pegnyemb et al., 2001, 2005). The large distribution of amentoflavone isolated in the preceding steps, suggests it could be a taxonomic marker of the genus *Ouratea* (Felicio et al., 2004), whereas lophirone A occurs in almost all genera of the family: *Lophira alata* (Ghogomu et al., 1987), *Ochna afzelii* and *Ouratea sulcata* (Pegnyemb et al., 2001, 2005) and in this study. Lanceolin C,

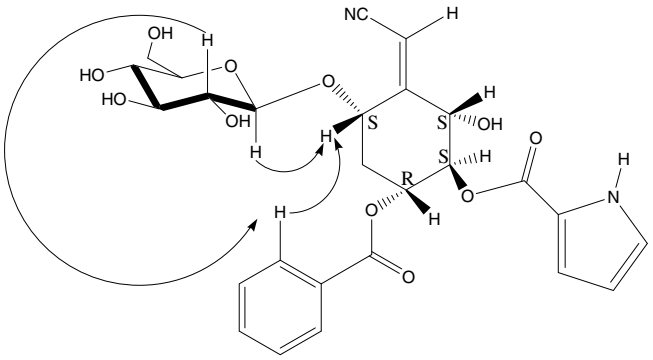


Fig. 4. Characteristic NOESY correlations of campyloside B (**2**).

Table 3
Minimal inhibitory concentration of the crude extracts of *C. glaucum* and *O. turnarea* against Gram-positive cocci

Gram-positive cocci	Minimal inhibitory concentration		
	C. glaucum (mg/ml)	*O. turnarea* (mg/ml)	Gentamicin (μg/ml)
Enterococcus hirae ATCC 9790	5	5	16
Enterococcus sp. P054	2.5	5	2
Staphylococcus aureus ATCC 25923	2.5	5	16
Staphylococcus aureus U271	2.5	5	<0.125
Staphylococcus saprophyticus	1.25	2.5	<0.125

lanceolin A and lanceolin B have been reported from sister species *L. alata* and *Lophira lanceolata*; nitrile glycosides with an analogue molecular mass like campyloside A can offer at this stage an additional approach to the taxonomy within the Ochnaceae family.

2.4. Biological assays on the samples

The use of *C. glaucum*, and *O. turnarea* in folk medicine has not yet been reported. In order to evaluate their antimicrobial properties, *in vitro* antimicrobial tests were carried out on the crude extracts of these two species, on serotobenine, campylosides A (**1**) and B (**2**). The crude extracts of these plants showed good activity against Gram-positive cocci (*Enterococcus* sp. P054, *Enterococcus hirae* ATCC 9790, *Staphylococcus aureus* ATCC 25923, *S. aureus* U271 and *Staphylococcus saprophyticus*). The minimal inhibitory concentration of these crude extracts varies from 1.25 to 5 mg/ml (Table 3). There were no activities against Gram-negative bacilli (*Escherichia coli*, *Klebsiella* spp. *Serratia marsescens*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) or fungi (*Candida* spp., *Cryptococcus neoformans* sero D, *Aspergillus* spp., *Tricophyton* spp.). The extract with the greatest antimicrobial activity was that of *C. glaucum*. Neither serotobenine nor campylosides A (**1**) and B (**2**) exhibited any activity on the same bacterial strains. However, serotobenine has exhibited particular antioxidant and antibacterial activities in other conditions (Kumarasamy et al., 2002).

3. Experimental

3.1. General

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. NMR spectra were run on a Bruker instrument equipped with a 5 mm ^{1}H and ^{13}C probe operating at 400 and 100 MHz, respectively, with TMS as internal standard. ^{1}H assignments were made using 2D-COSY and NOESY (mixing time 500 ms) while ^{13}C assignments were made using 2D-HSQC and HMBC experiments. For this latter, the delay was 70 ms. Melting points were measured on a Büchi apparatus and are uncorrected. IR data were measured on a JASCO FTIR-300E spectrometer with KBr pellets. Silica gel 70–230 mesh (Merck) and Sephadex LH-20 were used for column chromatography while precoated aluminium sheets silica gel 60 F_{254} were used for TLC. The HR-ESI mass spectra were run on an Applied Biosystems API Q-STAR PULSAR. The solvent systems were (I) CH_2Cl_2/MeOH:50/1, (II) CH_2Cl_2/MeOH:20/1, (III) CH_2Cl_2/MeOH:15/1, (IV) CH_2Cl_2/MeOH (10/1), (V) CH_2Cl_2/MeOH:95/5% and (VI) 100% MeOH.

3.2. Plant materials

The roots of *C. glaucum* (Tiegh) Farron and *O. turnarea* (Hook.) Hutch & Dalz were collected, respectively, at Sok Elle (December 2004) and Ntui (April 2006) in Centre-Cameroon. All these plant materials were identified by Mr. Nana Victor (botanist). The voucher samples (No. 28192/SRF/CAM, No. 10134/SRF/CAM), respectively, were deposited at the National Herbarium in Yaoundé, Cameroon.

3.3. Extraction of plant materials

Dried stem material of *C. glaucum* were ground and the resulting powder (1.45 kg) was extracted with MeOH during 48 h at room temperature. After filtration and removal of solvent, the solid product (230 g) was submitted to a new extraction using CH_2Cl_2–MeOH (1:1) to yield 122 g of a crude extract of which 92 g were analysed by chromatography and 10 g of the residue allowed to antimicrobial assays.

By using the same process, 2.3 kg of *O. turnarea* subjected to extraction with MeOH produced 120 g of a crude extract after a new extraction in the mixture CH_2Cl_2–MeOH (1:1).

3.4. Isolation of campyloside A (**1**)

The analysis of the crude extract of *C. glaucum* by a CC of SiO_2 using CH_2Cl_2–MeOH as eluent with increasing polarity systems (from 50:1 to pure MeOH) gave five fractions after monitoring and combination by TLC. Fraction 3 (8 g), indexed CG_3, produced three sub-fractions after a CC of SiO_2 gel with CH_2Cl_2/MeOH:15/1. The first sub-fraction, CG_{3a} (2 g) was purified by a CC of SiO_2 gel with CH_2Cl_2/MeOH:15/1 to yield campyloside A, **1** (38 mg) and lophirone A (14 mg). Fraction 4 (CG_4, 14 g) produced amentoflavone (21 mg) after successive CC on SiO_2 gel and Sephadex LH-20.

3.5. Isolation of campyloside B (**2**)

The third sub-fraction from CG_3 mentioned above which was indexed CG_{3c} (3 g) yielded campyloside B, **2** (10 mg) after a CC of SiO_2 with pure MeOH. Moreover, the fifth fraction indexed CG_5 (31 g) yielded **2** (30 mg) after successive CC of Sephadex LH-20 using MeOH as eluent and CC of SiO_2 gel with CH_2Cl_2/MeOH:10/1.

3.6. Fractionation of O. turnarea crude extract

Flash chromatography using CH_2Cl_2, CH_2Cl_2/MeOH and MeOH as eluents gave six main fractions (T_1: 4.8 g, T_2: 20.1 g, T_3: 3.6 g, T_4: 12.0 g, T_5: 5.6 g and T_6: 30.3 g).

Fraction T_2 was submitted to a silica gel CC and five sub-fractions (T_{2a}: 0.5 g, T_{2b}: 1.2 g, T_{2c}: 1.3 g, T_{2d}: 4.0 g and T_{2e}: 2.2 g) were obtained. T_{2d} was chromatographed over Sephadex LH-20 (MeOH) and purified by preparative TLC (CH_2Cl_2/MeOH:10/1) rendering serotobenine (35 mg) and lophirone A (19 mg). Fraction T_4 was subjected to silica gel CC to yield four sub-fractions (T_{4a}: 0.9 g, T_{4b}: 0.5 g, T_{4c}: 3.2 g and T_{4d}: 1.7 g). Purification of T_{4a} over silica gel with mixture (CH_2Cl_2/MeOH:10/1) yielded amentoflavone (11.0 mg).

T_{4c} was subjected to repeated chromatography over Sephadex LH-20 (MeOH) to yield lophirone C (16 mg), isolophirone C (9 mg). Purification of T_{4d} by repeated chromatography over Sephadex LH-20 and preparative TLC (CH_2Cl_2/MeOH:8/1) afforded calodenin B (12 mg).

3.7. Campyloside A (**1**) $C_{21}H_{25}O_{10}N$

White solid; $[\alpha]_D^{25}$ −28° (c 0.1, MeOH); m.p. 298–299 °C; IR υ_{max}^{KBr} cm^{-1}: 3320, 2215, 1718, 1623, 1602, 1501; TLC Rf: 0.33 (CH_2Cl_2/MeOH:95/5); ESI MS *m*/*z*: 452.162 $[M+H]^+$ (calcd. for

$C_{21}H_{26}O_{10}N$ 452.156); for ^1H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectral data (Table 1).

3.8. Campyloside B (**2**) $C_{26}H_{28}O_{11}N_2$

White crystal; $[\alpha]_D^{25}$ −30° (c 0.1, MeOH); m.p. 281–282 °C; IR υ_{max}^{KBr} cm^{-1}: 3320, 2215, 1718, 1623, 1602, 1501; TLC Rf: 0.29 (CH_2Cl_2/MeOH:95/5); ESI-MS *m/z*: 545.180 $[M+H]^+$ (calcd. for $C_{26}H_{29}O_{11}N_2$ 545.177); for ^1H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) spectral data, see Table 2.

3.9. Antimicrobial assays

The antimicrobial activity of each crude extract and each compound was measured *in vitro* against 30 microbial cultures representing 5 Gram-positive cocci, 13 Gram-negative bacilli and 12 fungi (6 yeasts and 5 filamentous fungi). Each extract and compounds were dissolved in MeOH–H_2O (1:1) to give 200 mg/ml crude extract and 1 mg/ml compounds. The antimicrobial activities of each diluted extract and compounds were then investigated by disc-diffusion methods, as recommended by the National Committee of Laboratory Standards. The minimal inhibitory concentrations (MIC) of each extract, against each microbial species that provided inhibition zone more than 15 mm with the extract in the disc-diffusion assays, were then determined using an agar-dilution method. The MIC was considered as the lowest concentration of an extract at which no visible growth was observed. As controls, the MIC of gentamicin and econazole against each bacterial species and fungi, respectively, were similarly determined. These assays were conducted as described previously (Gangoue-Pieboji et al., 2006).

Acknowledgements

We acknowledge support by International Foundation for Science (IFS), Stockholm, Sweden, and the Organization for Prohibition of Chemical Weapons (OPCW), The Hague, The Netherlands, through a Grant to Pr. Pegnyemb (No. F/3330-2F). We thank Mr. Nana Victor (National Herbarium of Cameroon) for his assistance in collection and identification of the plant material, Dr. J.P. Brouard and Mr. L. Dubost for the mass spectra. The authors are also grateful to the University of Yaounde I Grant committee and the French "Ministère de l'Education Nationale" for financial assistance.

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