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A dihydrochalcone derivative and further steroidal saponins from *Sansevieria trifasciata* Prain

DOI 10.1515/znc-2017-0027
Received February 20, 2017; revised April 13, 2017; accepted April 25, 2017

Abstract: Phytochemical investigation of the aerial parts of *Sansevieria trifasciata*, one of the most common Dracaenaceae plants, has resulted in the isolation of a new dihydrochalcone derivative named trifasciatine C (**1**), four previously unreported steroidal saponins as two pairs of inseparable regioisomers: trifasciatosides K/L (**2/3**), M/N (**4/5**), together with the known 1,2-(dipalmitoyl)-3-*O*-*β*-D-galactopyranosylglycerol (**6**), aconitic acid (**7**), and 1-methyl aconitic acid (**8**). Their structures were elucidated mainly by extensive spectroscopic analysis (1D and 2D nuclear magnetic resonance) and high-resolution electrospray ionization-mass spectrometry, as well as chemical methods and comparison of their spectral data with those of related compounds. Compounds **2/3** and **4/5** were evaluated for their antiproliferative activity on Hela cells, and no significant effect was observed.

Keywords: dihydrochalcone; Dracaenaceae; *Sansevieria trifasciata*; steroidal saponins.

1 Introduction

Sansevieria trifasciata Prain belongs to the Draceanaceae family which includes more than 60 species distributed in tropical and subtropical dry climate regions throughout the world [1, 2]. In South Africa and tropical America, *S. trifasciata* has been used in traditional medicine for the treatment of inflammatory conditions and sold as a crude drug in the market to treat victims of snake bites [3]. Previous phytochemical investigation of the EtOAc soluble fraction of the MeOH extract of this plant led to the isolation of two new minor homoisoflavonoids (trifasciatines A and B) [4], while the *n*-butanol fraction yielded steroidal saponins [5, 6]. In our continuous search for bioactive secondary metabolites from Cameroonian medicinal plants, we undertook extensive phytochemical investigation of the EtOAc soluble fraction of the MeOH extract of this plant. The present paper describes the isolation and structure elucidation of one new dihydrochalcone derivative trivially named trifasciatine C (**1**) and two pairs of previously unreported steroidal saponins named trifasciatosides K and L (**2**, **3**) and trifasciatosides M and N (**4**, **5**) obtained as inseparable regioisomers.

2 Results and discussion

The EtOAc soluble fraction of the methanol extract of *S. trifasciata* was subjected to column chromatography on silica gel and Sephadex LH-20 to afford one previously undescribed dihydrochalcone derivative named trifasciatine C (**1**) and two pairs of new steroidal saponins named trifasciatosides K and L (**2**, **3**) and trifasciatosides M and N (**4**, **5**) obtained as inseparable regioisomers (Figure 1), together with the known 1,2-(dipalmitoyl)-3-*O*-*β*-*D*-galactopyranosylglycerol (**6**) [7], aconitic acid (**7**) [8] and 1-methyl aconitic acid (**8**) [8].

Trifasciatine C (**1**) was isolated as a yellowish gum from hexane-EtOAc (1-1). Its high-resolution electronspray ionization-mass spectrometry (HRESIMS) exhibited a pseudomolecular ion peak ($[M+Na]^+$) at *m/z* 369.0960, corresponding to the molecular formula $C_{18}H_{18}O_7$ (calc. for $C_{18}H_{18}O_7Na$: 369.0945). The IR spectrum of **1** showed characteristic absorption bands for hydroxy (3440 cm^{-1}) and carbonyl (1643 cm^{-1}) groups. In the proton nuclear magnetic resonance (^1H NMR) spectrum, the signals at δ_H 7.49 (d, $J=9.2$, H-6′) and 6.38 (d, $J=9.2$, H-5′) (Table 1) suggested a pair of *ortho*-coupled protons in ring A [4, 9]. The substitution pattern of ring B was shown to be an ABX system

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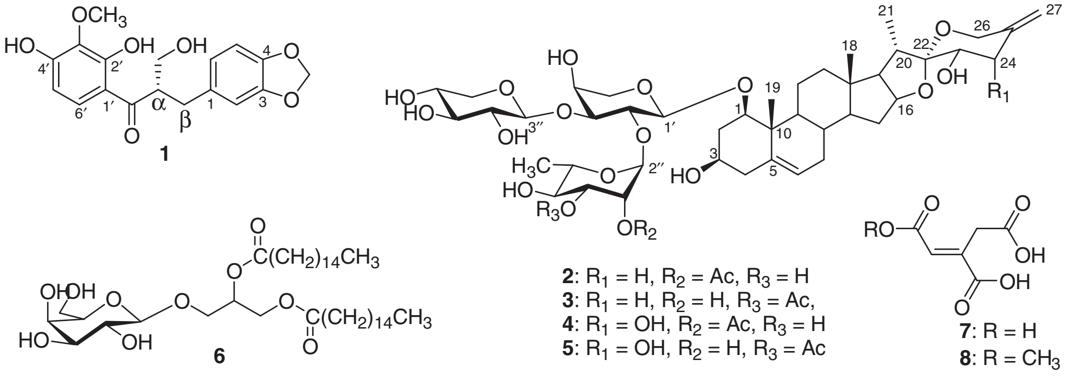

Figure 1: Structures of compounds **1–8** isolated from *S. trifasciata*.

Table 1: ^{1}H and ^{13}C NMR data (700 and 175 MHz, respectively, CD_3OD) of compound **1**.

Position	δ_H	δ_C
C=O	–	nd
α	3.88 (m)	50.1
β	2.83 (dd, *J* = 13.7, 5.3); 2.91 (dd, *J* = 13.7, 8.1)	34.8
1	–	132.9
2	6.69 (d, *J* = 1.4)	108.3
3	–	148.0
4	–	148.4
5	6.66 (d, *J* = 7.9)	107.4
6	6.63 (dd, *J* = 7.9, 1.4)	121.6
1′	–	114.2
2′	–	158.0
3′	–	136.5
4′	–	161.6
5′	6.38 (d, *J* = 9.2)	107.6
6′	7.49 (d, *J* = 9.2)	127.0
$-CH_2OH$	3.90 (m); 3.70 (m)	62.8
$-OCH_2O-$	5.90 (dd, *J* = 4.8, 1.2)	100.6
–OMe	3.83 (s)	59.1

nd, Not determined.

with signals at δ_H 6.66 (d, *J* = 7.9, H-5), 6.69 (d, *J* = 1.4, H-2), and 6.63 (dd, *J* = 7.9, 1.4, H-6). The ^{1}H NMR spectrum also showed a signal integrating for two protons at δ_H 5.90 suggesting a methylenedioxyl (OCH_2O) moiety [9, 10] and a singlet of three protons at δ_H 3.83 suggesting the presence of a methoxyl group in **1**. In addition, the presence of two couples of geminal protons resonating at δ_H [3.70 (m, CH_2O) and 3.90 (m, CH_2O)] and [2.83 (dd, *J* = 13.7, 5.3, H_a-β) and 2.91 (dd, *J* = 13.9, 8.1, H_b-β)], as well as an aliphatic proton signal at δ_H 3.88 (H-α), revealed that compound **1** was related to 3-benzylchroman-4-one type homoisoflavonoids [4, 9, 10]. The ^{13}C NMR spectrum exhibited 17 signals including those of 12 aromatic carbons, three methylenes, one sp3 methine, and one methyl signal. Extensive analysis of ^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple-bond correlation spectroscopy (HMBC) spectra allowed us to assign ^{1}H and ^{13}C resonances (Figure 2). However, the chemical shift of the oxymethylene carbon resonating at δ_C 62.8 in **1**, in comparison with the downfield shift observed for the related carbon in trifasciatine A (δ_C 71.3) previously isolated from this plant [4], suggested that **1** was an opened C-ring homoisoflavonoid derivative. This was also confirmed by the lack of HMBC correlations between the geminal protons resonating at δ_H 3.70 (CH_2O) and 3.90 (CH_2O) and the carbon at δ_C 158.0 (C-2′) as well as the presence of one additional oxygen atom in **1** compared to trifasciatine A depicted from the mass data. The location of the methylenedioxyl group was confirmed by the HMBC correlations observed between its protons at δ_H 5.90 with the *O*-bearing aromatic carbons at δ_C 148.0 (C-3) and 148.4 (C-4) (Figure 2). Furthermore, the cross peak correlation observed between the methoxy proton signal at δ_H 3.83 (OMe) and the carbon at δ_C 136.5 (C-3′) showed that it was linked at C-3′. Biogenetically, compound **1** could have derived from trifasciatine A previously isolated from the same plant due to the enzymatic cleavage of its C-ring since it was shown that etherase enzymes produced by some bacteria are able to break ether bonds [11]. The configuration at C-α was proposed to be *S* based on the positive sign of its optical rotation by opposition to that of C-3 in trifasciatine A whose configuration was recently determined by some of us using electronic circular

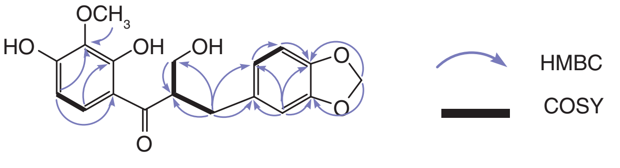

Figure 2: Selected HMBC and ^{1}H-^{1}H COSY correlations for compound **1**.

dichroism [4]. Consequently, the structure of **1** was elucidated as (α*S*) α-hydroxymethyl-2′,4′-dihydroxy-3′-methoxy-3,4-methylenedioxydihydrochalcone to which we gave the trivial name trifasciatine C.

Table 2: ^{13}C NMR data (175 MHz, CD_3OD) of compounds **2–5** (δ in ppm).

Position	2	3	4	5
1	83.4	83.6	83.4	83.5
2	35.2	35.5	35.8	36.3
3	68.5	68.7	68.4	68.6
4	42.2	42.3	42.0	42.1
5	138.1	138.1	138.0	138.1
6	124.5	124.5	124.6	124.5
7	32.2	32.5	31.4	31.3
8	33.4	33.6	32.5	32.5
9	50.0	50.1	49.7	49.9
10	42.0	42.1	42.0	42.0
11	23.3	23.3	23.3	23.3
12	40.1	40.1	40.0	40.1
13	40.5	40.3	40.5	41.1
14	56.5	56.7	56.2	56.4
15	32.5	32.6	32.5	31.8
16	82.5	82.4	83.0	82.9
17	61.8	62.0	61.8	62.5
18	15.7	15.8	15.7	15.8
19	15.1	15.0	16.0	16.8
20	35.9	36.9	35.4	35.7
21	13.9	13.9	13.0	13.9
22	110.6	110.6	111.6	112.1
23	68.7	68.9	69.7	69.7
24	38.5	38.7	74.2	74.2
25	143.1	143.1	144.6	144.5
26	63.4	63.4	60.0	59.7
27	108.5	108.5	112.0	112.0
Ara				
1′	99.5	99.6	99.6	99.5
2′	74.1	74.3	73.3	74.0
3′	84.6	84.2	84.0	84.2
4′	69.3	69.6	69.2	69.5
5′	67.1	67.4	67.8	67.7
Rha				
1″	97.0	100.2	97.1	100.2
2″	72.5	68.6	72.5	68.7
3″	69.1	74.2	69.1	74.5
4″	73.8	69.0	73.5	69.7
5″	68.4	69.7	68.0	67.6
6″	16.4	17.1	17.0	17.1
Xyl				
1‴	104.3	104.9	104.9	104.3
2‴	74.4	74.6	74.2	74.1
3‴	76.4	76.6	76.0	76.2
4‴	71.0	71.2	69.9	70.1
5‴	66.9	66.5	67.0	66.4
Ac	171.3	171.4	171.3	171.4
	19.7	20.1	19.7	19.7

Trifasciatosides K/L (**2/3**), isolated as a white amorphous powder from MeOH, had the molecular formula $C_{45}H_{68}O_{18}$, as determined from its HRESIMS which showed the pseudomolecular ion peak at *m/z* 919.4387 ($[M+Na]^+$) (calcd for $C_{45}H_{68}O_{19}Na$: 919.4298). The IR spectrum exhibited a broad absorption band due to hydroxyl groups at 3300 cm^{-1}, as well as a strong absorption due to carbonyl groups at 1724 cm^{-1}. It was shown to be a mixture of two monoacetylated spirostanol saponins **2** and **3** from the interpretation of the NMR data. The ^{1}H NMR spectrum showed two tertiary methyl signals at δ_H 1.12/1.13 (H-18) and 1.09/1.10 (H-19) (each s), two secondary methyl signals at δ_H 1.27/1.30 (d, $J=6.2$, H-6″), exomethylene proton signals at δ_H 4.79 (H-27a) and 4.83 (H-27b), the olefinic proton signal at δ_H 5.58 (brd, $J=5.1$, H-6), and three anomeric proton signals at δ_H 4.35/4.37 (d, $J=7.3$, H-1′), 4.40/4.41 (d, $J=6.0$, H-1‴), and 5.32/5.40 (brs, H-1″). The ^{13}C NMR spectrum exhibited signals characteristic of 1,3,23-trihydroxyspirosta-5,25(27)-diene [5, 6] at δ_C 83.4/83.6 (C-1), 68.5/68.7 (C-3), 138.1(C-5), 124.5 (C-6), 82.5/82.4 (C-16), 68.7/68.9 (C-23), 143.1 (C-25), and 108.5 (C-27) (Table 2). Assignment of the other proton and carbon signals of the aglycone part was achieved by careful examination of the HSQC, HMBC, and ^{1}H-^{1}H COSY spectra. Evaluation of spin-spin couplings and chemical shifts in the sugar part allowed the identification of one 2,3-disubstituted arabinopyranosyl unit, one terminal rhamnopyranosyl residue, and one xylopyranosyl unit in each compound. In addition, **2/3** afforded arabinose, xylose, and rhamnose on acid hydrolysis. Their absolute configurations were determined by gas chromatography (GC) analysis of the corresponding trimethylsilated L-cysteine adducts [12]. The presence of acetyl groups was indicated by methyl singlets at δ_H 2.12/2.13 (*s*, MeCO), whereas in the ^{13}C NMR spectrum, the signals of ester carbonyls at δ_C 171.3/171.4 (MeCO) and methyl carbons at δ_C 19.7/20.1 (MeCO) were observed. The ^{1}H-^{1}H COSY spectrum allowed the resonances observed at a lower field at δ_H 5.14 (dd, $J=3.6$; 1.6) and 4.94 (dd, $J=10.0$; 3.2) to be assigned to H-2″ and H-3″ of the two rhamnopyranosyl units, respectively, which suggested that they were acetylated. This was confirmed by HMBC correlations between H-2″ and H-3″ and the acetyl carbonyl carbons at δ_C 171.3 and 171.4, respectively. The HMBC correlations between the anomeric proton signals at δ_H 4.40/4.41 (d, $J=6.0$, H-1‴) and the carbon at δ_C 84.2/84.6 (C-3′), 5.40 (brs, H-1″) and 74.1 (C-2′), 5.32 (d, $J=1.8$, H-1″) and 74.3 (C-2′), and finally between the protons at δ_H 4.37/4.35 (d, $J=7.3$, H-1′) and the carbons at δ_C 83.4/83.6 (C-1) proved the sequence of the sugar chain at C-1 to be α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranosyl in both compounds. Based on the above data, **2/3** was elucidated as a mixture of (23*S*)-3β,23-dihydroxyspirosta-5,25(27)-dien-1β-yl *O*-(2-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside (Trifasciatoside K) and (23*S*)-3β,23-dihydroxyspirosta-5,25(27)-dien-1β-yl*O*-(3-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside (Trifasciatoside L).

The positive HRESIMS of compounds **4/5** exhibited a pseudomolecular ion peak at *m/z* 935.4335 ([M + Na]$^+$) corresponding to the molecular formula $C_{45}H_{68}O_{19}$ with 16 mass units more than **2/3**. The ^{1}H NMR spectrum exhibited one signal with regard to H-21 at δ_H 0.95 (d, $J = 7$), two signals for H-18 at δ_H 1.12 (s) and 1.11 (s), one signal due to H-19 at δ_H 0.90 (s) together with the olefinic proton signal at δ_H 5.55 (brs, H-6), and the exomethylene proton signals H-27 at δ_H 4.98 (brs) and 5.06 (brs). The ^{13}C NMR spectrum showed signals characteristic of 1,3,23,24-tetrahydroxyspirosta-5,25(27)-diene [5] at δ_C 83.4/83.6 (C-1), 68.4/68.6 (C-3), 138.0/138.1 (C-5), 124.6/124.5 (C-6), 83.0/82.9 (C-16), 69.7 (C-23), 74.2 (C-24), 144.6/144.5 (C-25), and 112.0 (C-27) (Table 2). GC analysis of chiral derivatives of sugars from acid hydrolysate of **4/5** showed the presence of L-rhamnose, D-xylose, and L-arabinose [12]. Comparison of the ^{1}H and ^{13}C NMR spectra of **4/5** with those of **2/3** revealed that the structures of the rings A–E portion and sugar moiety (including the acetylation patterns) attached at C-1 of the aglycone were identical. However, significant differences were recognized in the signals from the ring F portion (C-22 to C-27) (Table 2). This indicated that the only difference between the above compounds is the presence of a hydroxyl group on ring F in **4/5**. The location of this OH group was determined by using a combination of ^{1}H, ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, and HMBC spectra. HMBC correlations were depicted between the proton signal at δ_H 3.76 (d, $J = 3.2$, H-23) and the carbon signal at δ_C 111.6 (C-22) and between the proton signal at δ_H 4.22 (d, $J = 3.2$, H-24) and the carbons at δ_C 144.6 (C-25) and 112.0 (C-27), respectively. The structures of **4/5** were elucidated as (23*S*,24*S*)-3β,23,24-trihydroxyspirosta-5,25(27)-dien-1β-yl *O*-(2-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside and (23*S*,24*S*)-3β,23,24-trihydroxyspirosta-5,25(27)-dien-1β-yl *O*-(3-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside to which we gave the trivial named trifasciatosides M and N, respectively.

Since some steroidal saponins were shown to exhibit potent cytotoxicity [5, 13, 14], compounds **2/3** and **4/5** were evaluated for their antiproliferative activity on Hela cells, and no considerable effect was observed.

In conclusion, phytochemical investigation of the EtOAc extract of *S. trifasciata* has resulted in the isolation of a new dihydrochalcone derivative named trifasciatine C and four previously unreported monoacetylated steroidal saponins obtained as two pairs of inseparable regioisomers: trifasciatosides K/L (**2/3**) and M/N (**4/5**). It has been established that acetyl saponins are typical unstable compounds extensively distributed in many plant species, and this structural stability depends on different solvents and separation materials used for their purification [15, 16]. Therefore, it could be assumed that compound **3** (or **5**) could have resulted from compound **2** (or **4**) and vice versa from the migration of the acetyl group from 2 to 3 positions (vice versa) of the rhamnose moiety as shown by Zeng et al. [16], thus resulting to the two inseparable mixtures **2/3** and **4/5**. Although the isolated saponins exhibited no significant antiproliferative activity, the present work indicated that *S. trifasciata* represents a potential source of new steroidal saponins and phenolic compounds. Given the biological importance and the various uses of this medicinal plant, the isolated compounds will be screened for other biological activities in our future investigation.

3 Experimental section

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter (Perkin-Elmer, Waltham, MA, USA). IR spectra were recorded on a Shimadzu infrared spectrometer (Shimadzu, Kyoto, Japan). UV spectra were registered on a Photolab 6600 UV-VIS spectrophotometer (Orbit Technologies Pvt. Ltd., India), and HRESIMS were recorded on an Agilent 6210 ESI-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). GC-MS analysis was performed on a GCMS-QP2010SE (Shimadzu, Japan) with inert cap 5MS/Sil i.d.0.25 × 30 m (GL Sciences Inc., Japan) [Column temperature: 100–280 °C, rate of temperature increase: 10 °C/min]. The following sugar samples and reagents were commercially obtained: L-rhamnose, D-rhamnose (Funakoshi Co., Ltd., Japan), D-xylose, L-xylose (Wako Pure Chemical Industries, Ltd., Japan), D-arabinose, L-arabinose (Kishida Chemical Co., Ltd., Japan), L-cysteine methyl ester hydrochloride (Kanto Chemical Co., Inc., Japan), and *N*-trimethylsilylimidazole (TMS-imidazole) (Tokyo Kasei Kogyo Co., Ltd., Japan). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 700 spectrometer (Bruker BioSpin, Billerica, MA, USA) (700 MHz for ^{1}H and 175 MHz for ^{13}C) in deuterated MeOH. All chemical shifts (δ) are given in ppm with reference to the residual solvent signal and coupling constants (*J*) are in Hz. Thin-layer chromatography was carried out on precoated silica gel 60 F254 (Merck, Darmstadt, Germany) plates developed with hexane:EtOAc, EtOAc:MeOH, and EtOAc:MeOH-H_2O. They were visualized under UV light (254 and 365 nm) and by spraying with 10% aqueous H_2SO_4 followed by heating.

3.2 Plant material

The aerial part of *S. trifasciata* was collected in Dschang in March 2014 and authentified at the Cameroon National Herbarium, Yaounde, where voucher specimens are deposited (No 43509/HCN).

3.3 Extraction and isolation

The powdered aerial parts of *S. trifasciata* (3.3 kg) were extracted three times with MeOH (3 × 10 L) at room temperature, and the filtrate obtained was evaporated under reduced pressure to yield the crude MeOH extract (497.3 g, 15% yield). Part of this extract (450 g) was triturated with EtOAc to yield 102 g of the EtOAc soluble fraction. About 97 g of the EtOAc soluble fraction was submitted to a silica gel column chromatography eluted with hexane:EtOAc and EtOAc:MeOH with increasing polarity yielding several fractions. The fraction eluted with hexane:EtOAc 6:4 (1.2 g) was subjected to Sephadex LH-20 (Merck, Darmstadt, Germany) column chromatography eluted with MeOH to give a mixture (30 mg) which was repeatedly chromatographed on silica gel (hexane:EtOAc 65:35) to afford **1** (2.1 mg). The fraction eluted with hexane:EtOAc 2:8 (3.0 g) was subjected to Sephadex LH-20 column chromatography eluted with MeOH to give a subfraction which was recrystallized to yield **6** (15 mg). The fraction eluted with EtOAc:MeOH 90:10 (3.8 g) was purified on a Sephadex LH-20 column eluted with MeOH and was repeatedly chromatographed on silica gel column (eluted with EtOAc:MeOH:H_2O 90:10:5) to afford **2/3** (10 mg) and **4/5** (7 mg), respectively. Recrystallization of the fraction eluted with EtOAc:MeOH 85:15 (15 g) yielded **7** (110 mg) and **8** (25 mg).

Trifasciatine C (**1**): Yellowish gum. – $[\alpha]^{25}_{D} = +20$ ($c = 0.3$, MeOH). – UV (MeOH): λ_{max} (log ε) = 291 (3.17). – IR (NaCl): $\nu_{max} = 3400, 2920, 1643, 1245$ cm^{-1}. – ^{1}H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz): see Table 1. – HRESIMS: *m/z* 369.0960 $[M+Na]^+$ (calcd. for $C_{18}H_{18}O_7Na$: 369.0945).

Trifasciatosides K/L (**2/3**): Amorphous powder. – $[\alpha]^{25}_{D} = -30$ ($c = 0.8$, MeOH). – IR (KBr): $\nu_{max} = 3300$, 2972, 1724, 1446, 1373, 1253, 1137, 1048, 981 cm^{-1}. – ^{1}H NMR (CD_3OD, 700 MHz): 5.58 (brd, *J* = 5.1, H-6), 5.40 (brs, H-1″), 5.32 (d, *J* = 1.8, H-1″), 5.14 (dd, *J* = 3.6, 1.6, H-2″), 4.94 (o, H-3″), 4.83 (brs, H-27), 4.79 (brs, H-27), 4.40/4.41 (d, *J* = 6.0, H-1‴), 4.35/4.37 (d, *J* = 7.3, H-1′), 3.42 (m, H-3), 1.27/1.30 (d, *J* = 6.2, H-6″), 1.12/1.13 (s, H-18), 1.09/1.10 (s, H-19), 0.95 (d, *J* = 7.0, H-21). – ^{13}C NMR (CD_3OD, 175 MHz,): see Table 2. – HRESIMS: 919.4387 $[M+Na]^+$ (calcd. for $C_{45}H_{68}O_{18}Na$: 919.4303).

Trifasciatosides M/N (**4/5**): Amorphous powder. – $[\alpha]^{25}_{D} = -23.3$ ($c = 0.3$, MeOH). – IR (KBr): ν_{max} – 3330, 2921, 1726, 1377, 1253, 1047 cm^{-1}. – ^{1}H NMR (CD_3OD, 700 MHz): 5.55 (brs, H-6), 5.40 (d, *J* = 1.0, H-1″), 5.32 (d, *J* = 1.7, H-1″), 5.15 (dd, *J* = 3.5, 1.5, H-2″), 5.06 (brs, H-27), 4.90 (o, H-3″), 4.98 (brs, H-27), 4.42/4.44 (d, *J* = 6.3, H-1‴), 4.37/4.33 (d, *J* = 7.3, H-1′), 3.34 (m, H-3), 1.27/1.24 (d, *J* = 6.1, H-6″), 1.11/1.12 (s, H-18), 0.95/0.92 (d, *J* = 7.0, H-21), 0.90/0.91 (s, H-19). – ^{13}C NMR (CD_3OD, 175 MHz): see Table 2. HRESIMS: 935.4335 $[M+Na]^+$ (calcd. for $C_{45}H_{68}O_{19}Na$: 935.4247).

3.4 Acid hydrolysis and GC-MS

Compounds **2/3** and **4/5** (ca. 1.0 mg) were individually heated in 1 M HCl (0.1 mL) at 90 °C for 3 h. Each reaction mixture was dried under reduced pressure and dissolved in pyridine (0.2 mL). TMS-imidazole (50 μL) was added to part of the solution (0.1 mL) then heated at 50 °C for 30 min. The reaction mixture was diluted with H_2O (0.2 mL) and extracted with *n*-hexane (0.1 mL) then analyzed by GC-MS by comparison with standard samples. L-cysteine methyl ester hydrochloride (ca. 1.0 mg) was added to the remaining pyridine solution (0.1 mL) and heated at 60 °C for 1 h, then the TMS derivative was prepared the same manner mentioned above and analyzed by GC-MS. Standard TMS-sugars (t_R, min.) were the following: TMS-xylose, $t_R = 12.08$, 12.68; TMS-rhamnose, $t_R = 11.06$, 11.88; and TMS-arabinose, $t_R = 10.87$, 10.92, 11.29. Standard TMS-thiazolidine derivatives (t_R, min.) were the following: D-xylose, $t_R = 17.49$; L-xylose, $t_R = 17.74$; D-rhamnose, $t_R = 18.25$; L-rhamnose, $t_R = 18.13$; D-arabinose, $t_R = 17.80$; and L-arabinose, $t_R = 17.48$.

3.5 Cell culture and cell proliferation assay

Human malignant epithelial cells (HeLa) were cultured in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (FBS) kept in an incubator at 37 °C in a humidified air containing 5% CO_2. FBS was purchased from Nichirei Bioscience Inc. (Tokyo, Japan). Cell viability was determined by a Cell-Titer 96

Aqueous Non-Radioactive Cell Proliferation (MTS) Assay (Promega, WI) according to the manufacturer's protocol. HeLa cells (1×10^4 cells/well) were seeded in 96 well plates and incubated for 24 h, subsequently grown with compounds for additional 48 h, and then cell proliferation assay was performed.

4 Supplementary data

HRESIMS, ^{1}H and ^{13}C NMR, COSY, HSQC and HMBC spectra for new compounds are available.

Acknowledgments: The authors are grateful to the Alexander von Humboldt Foundation (AvH), Bonn, Germany, for the financial support of this work through the equipment grant to Prof Dr. Tapondjou A. Léon.

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Supplemental Material: The online version of this article (DOI: 10.1515/znc-2017-0027) offers supplementary material, available to authorized users.