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Isolation, characterization and antimicrobial activities of polyacetylene glycosides from *Coreopsis tinctoria* Nutt.

Jia Guo [a], Ao Wang [a], Ke Yang [a], Hao Ding [a, b], Yimin Hu [a], Yumeng Yang [a], Siqi Huang [a], Jingguo Xu [a], Tianxing Liu [a], Haiyan Yang [b], Zhihong Xin [a, *]

[a] Key Laboratory of Food Processing and Quality Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing, 210095, People's Republic of China

[b] College of Food Science and Pharmacy, Xinjiang Agricultural University, Urumqi, People's Republic of China

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ABSTRACT

Polyacetylene glycosides, (6Z, 12E)-tetradecadiene-8,10-diyne-1-ol-3(*R*)-O-*β*-D-glucopyranoside (trivially named coreoside E) and (6Z, 12E)-tetradecadiene-8,10-diyne-1-ol-3(*R*)-O-*β*-L-arabinopyranosyl-(1 → 2)-*β*-D-glucopyranoside (trivially named coreoside F), were isolated from buds of *Coreopsis tinctoria* Nutt., together with one known compound, coreoside B. Their chemical structures were elucidated by extensive spectroscopic analysis and on the basis of their chemical reactivities. Coreoside E exhibited high levels of antimicrobial activity against *Staphylococcus aureus* and *Bacillus anthracis* with minimum inhibitory concentrations of 27 ± 0.27 and 18 ± 0.40 *μ*M, respectively, whereas coreoside F and coreoside B showed weak antimicrobial activity against *S. aureus* and *B. anthracis*.

1. Introduction

Polyacetylene glycosides are derivatives of polyacetylenes, a class of compounds that contain two or more carbon−carbon triple bonds in their carbon skeleton (Negri, 2015). Polyacetylene glycosides are widely distributed in a number of vegetables and medicinal plants. They are especially found in the families Asteraceae, Apiaceae, Araliaceae, and Campanulaceae, with representative examples such as *Daucus carota* L., *Apium graveolens* L., *Petroselinum crispum* Mill., *Panax ginseng*, and *Carthamus tinctorius*, *Atractylodes lancea* (Baranska et al., 2005; He et al., 2011; Ji et al., 2010; Lee et al., 2009; Silva et al., 2015; Zidorn et al., 2005). Previous studies reported that polyacetylene glycosides have a diverse range of biological effects, such as cytotoxicity (Park et al., 2002), anti-inflammatory (Zhang et al., 2013), antimicrobial (Pellati et al., 2006), anti-HIV (Zhang et al., 2002) and antiallergic (Wang et al., 2001) activities.

Coreopsis tinctoria Nutt. belongs to the Asteraceae/Compositae family and is a small, glabrous, aromatic annual plant that is distributed globally (Dias et al., 2010). It is known as “snow chrysanthemum” or “snow tea” in China, and grows on the Karakorum Mountains in southern Xinjiang at an altitude above 3000 m (Zhang et al., 2013). *C. tinctoria* contains a variety of bioactive phytochemicals, including phenolics, flavonoids, phenylpropanoids, sterols and polyacetylene glycosides (Ma et al., 2016; Wang et al., 2015; Zălaru et al., 2014). *C. tinctoria* is traditionally used in folk medicine to treat several diseases, such as hypertension, hyperlipidemia, diarrhea and internal pain (Dias et al., 2010; Zălaru et al., 2014). Although a few dozen papers have focused on the bioactivities of *C. tinctoria*, the precise chemical identification of the bioactives remain unclear.

The studies herein showed that the methanol extract of *C. tinctoria* buds exhibited significant antimicrobial activity against *S. aureus*, and this extract was further investigated. Two new (and one known) polyacetylene glycosides were isolated from the bioactive extract and evaluated for antimicrobial activity. Described are the separation process of the bioactives, and elucidation of their structures based on their spectroscopic properties and chemical reactivity.

* Corresponding author.
E-mail address: xzhfood@njau.edu.cn (Z. Xin).

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2. Results and discussion

2.1. Structural elucidation of the polyacetylene glycosides

Compound **1** was isolated as a brown amorphous powder. Its molecular formula was determined to be $C_{20}H_{28}O_7$ based on high-resolution electrospray ionization mass spectrometry (HRESIMS) analysis of the pseudomolecular ion peak at *m/z* 403.1742 $[M + Na]^+$ (calcd. 403.1757), indicating seven degrees of unsaturation. Its UV spectrum showed strong absorption bands at 230, 262, 276, 294 and 313 nm, which are characteristic of an ene-diyne chromophore (Zhou et al., 2006). Its IR spectrum displayed strong absorption bands at 3326, 2311 and 1603 cm^{-1}, corresponding to hydroxyl groups, triple bonds and double bonds, respectively (Zhou et al., 2006). These data suggested that **1** was an ene-diyne compound. The ^1H NMR spectroscopic data showed four olefinic protons at δ 6.34 (m, 1H), 5.65 (m, 1H), 5.61 (m, 1H) and 6.32 (m, 1H), an anomeric proton at δ 4.38 (d, $J = 7.84$ Hz, 1H), and several sugar or oxygenated protons between δ 3.1 and δ 4.0 (Fig. S1 and Table 1). The ^{13}C NMR spectrum of **1** (Fig. S2) showed the presence of 20 carbon signals. Six of these were assigned to a sugar moiety, including the anomeric carbon at δ 102.5 (C-1prime), with the remaining 14 assigned to the aglycone moiety, the latter corresponded to four acetylenic carbons at δ 78.9 (C-8), 71.8 (C-9), 71.7 (C-10), and 79.1 (C-11), four olefinic carbons at δ 148.2 (C-6), 108.4 (C-7), 109.4 (C-12), and 142.9 (C-13), two oxygenated carbons at δ 58.1 (C-1) and 76.5 (C-3), one methyl carbon at δ 17.4 (C-14), and three methylene carbons at δ 36.8 (C-2), 34.0 (C-4), and 28.6 (C-5), respectively. Taken together, these data indicated that **1** was a typical polyacetylene glycoside. Analysis of the $^1H-^1H$–COSY (Correlation Spectroscopy), HSQC (Heteronuclear Singular Quantum Correlation) and HMBC (Heteronuclear Multiple Bond Correlation) spectra of **1** allowed the proton and carbon resonances to be fully assigned (Fig. 1). Key correlations from H-1′ to C-3 and H-3 to C-1prime, were observed in the HMBC experiments, indicating that the sugar was connected directly to the C-3 position of the aglycone. The double bond between C-6 and C-7 was determined to be in a *cis* configuration, because of the strong NOESY correlation between H-6 and H-7, while the double bond between C-12 and C-13 was assigned a *trans* configuration on the basis of the NOESY correlation between H-12 and H-14. The sugar unit was identified as a β-glucopyranosyl group on the basis of its $^3J_{H\text{-}1',\ H\text{-}2'}$ coupling constant of 7.84 Hz. Acid hydrolysis of **1** with 1 M HCl liberated aglycone **1a** and D-glucose (Fig. 1), which were identified using gas chromatography-mass spectrometry (GC-MS) analysis of the corresponding trimethylsilyl L-cysteine derivatives that were directly compared with authentic samples of these materials prepared in the same manner. Both the sample prepared from **1** and D-glucose provided a compound with the same retention time of 17.82 min, indicating that compound **1** contained a D-glucose moiety. The absolute configuration of compound **1** at C-3 was assigned as *R* by comparison of its $[\alpha]_D$ value (−10.2°, c = 1.08, MeOH) with that of reference data (Umeyama et al., 2010). Based on these results, compound **1** was elucidated to be (6Z, 12E)-tetradecadiene-8,10-diyne-1-ol-3(*R*)-O-β-D-glucopyranoside, and trivially named coreoside E (Fig. 1).

Compound **2** was isolated as a brown amorphous powder and its molecular formula was determined to be $C_{25}H_{36}O_{11}$ based on HRESIMS analysis of the pseudomolecular ion peak at *m/z* 551.0295 $[M + K]^+$ (calcd. 551.0250). Its UV spectrum showed typical ene-diyne absorption bands at 230, 262, 276, 294 and 313 nm. Its IR spectrum had absorbtion corresponding to hydroxyl, carboxyl, olefinic and glycosidic functionalities at 3425, 1719, 1653 and 1023 cm^{-1}, respectively. Direct comparison of the ^1H and ^{13}C NMR data for **2** (Fig. S3 and Fig. S4) with that of **1** indicated that they shared the same skeleton, with the sole difference being the monosaccharide sugar unit of **1** was replaced by a disaccharide unit in **2**. This assignment was supported by the presence of two anomeric proton signals at δ 4.59 (d, $J = 6.72$ Hz, 1H), 4.49 (d, $J = 7.68$ Hz, 1H), and two corresponding anomeric carbon signals at 100.9 (C-1′), 104.2 (C-1″) (Table 1). The full structure of compound **2** was further confirmed by $^1H-^1H$ COSY, HSQC, and HMBC experiments. Key correlations from H-2′ to C-1″ and H-1″ to C-2′ were observed in the HMBC experiment, indicating that the second sugar unit was connected to the C-2′ position of the first sugar unit. The $^3J_{H\text{-}1',\ H\text{-}2'}$ and $^3J_{H\text{-}1'',\ H\text{-}2''}$ coupling constants of 6.72 and 7.68 Hz of the anomeric protons indicated that the two sugar units were β-glucopyranosyl and β-arabinopyranosyl groups, respectively. The absolute configurations of the two sugar units were identified as D-glucopyranosyl and L-arabinopyranosyl groups by the same procedures as used for compound **1**. The C-3 configuration was also assigned as *R* by comparison of the $[\alpha]_D$ value (−7.2°, c = 1.15, MeOH) with that of reference data (Umeyama et al., 2010). Consequently, compound **2** was determined as (6Z, 12E)-tetradecadiene-8,10-diyne-1-ol-3(*R*)-O-β-L-arabinopyranosyl-(1 → 2)-β-D-glucop-yranoside, and trivially named coreoside F (Fig. 1).

Compound **3** was also isolated as a brown amorphous powder. Its molecular formula was established as $C_{25}H_{36}O_{12}$ based on the HRESIMS analysis of the pseudomolecular ion peak at *m/z* 551.1372 $[M + Na]^+$ (calcd. 551.1360) and NMR data analysis. Its UV spectrum showed strong absorption bands at 239, 247, 278, 294 and 313 nm, indicating that **3** was also a polyacetylene glycoside. A careful comparison of the ^1H and ^{13}C NMR spectra of **2** and **3** indicated very similar signals, with the exception of a doublet corresponding to the 14-CH_3 proton of **2** at 1.83 (d, $J = 6.84$ Hz, 3H) being replaced by a hydroxymethyl signal at 4.05 (s, 1H) in the spectra of **3**.

Table 1
NMR spectroscopic data for compounds 1 and 2 in DMSO-d_6 at 400 (^1H) and 100 MHz (^{13}C).

	1		2	
	H	C	H	C
1a	3.68 (m, 1H)	58.1	3.61 (m, 1H)	58.0
1b	3.79 (m, 1H)		3.70 (m, 1H)	
2	1.79 (m, 1H)	36.8	1.77 (m, 1H)	36.6
3	3.89 (m, 1H)	76.5	3.95 (m, 1H)	76.2
4	1.71 (m, 1H)	34.0	1.73 (m, 1H)	33.7
5	2.33 (m, 1H)	28.6	2.30 (m, 1H)	28.6
6	6.34 (m, 1H)	148.2	6.32 (m, 1H)	148.3
7	5.65 (m, 1H)	108.4	5.64 (m, 1H)	109.4
8		78.9		79.1
9		71.8		71.7
10		71.7		71.7
11		79.1		78.9
12	5.61 (m, 1H)	109.4	5.64 (m, 1H)	108.3
13	6.32 (m, 1H)	142.9	6.32 (m, 1H)	142.9
14	1.84 (d, $J = 6.76$ Hz, 3H)	17.4	1.83 (d, $J = 6.84$ Hz, 3H)	17.4
Glc				
1′	4.38 (d, $J = 7.84$ Hz, 1H)	102.5	4.49 (d, $J = 7.68$ Hz, 1H)	100.9
2′	3.18 (t, $J = 8.20$ Hz, 1H)	73.9	3.26 (m, 1H)	81.3
3′	3.39 (m, 1H)	76.7	3.40 (m, 1H)	76.3
4′	3.30 (m, 1H)	70.2	3.36 (m, 1H)	70.0
5′	3.29 (m, 1H)	76.3	3.28 (m, 1H)	76.8
6′a	3.71 (m, 1H)	61.3	3.79 (m, 1H)	61.2
6′b	3.87 (m, 1H)		3.92 (m, 1H)	
Ara				
1″			4.59 (d, $J = 6.72$ Hz, 1H)	104.2,
2″			3.60 (m, 1H)	72.7
3″			3.53 (m, 1H)	68.0
4″			3.86 (m, 1H)	71.8
5″a			3.71 (m, 1H)	65.5
5″b			3.55 (m, 1H)	

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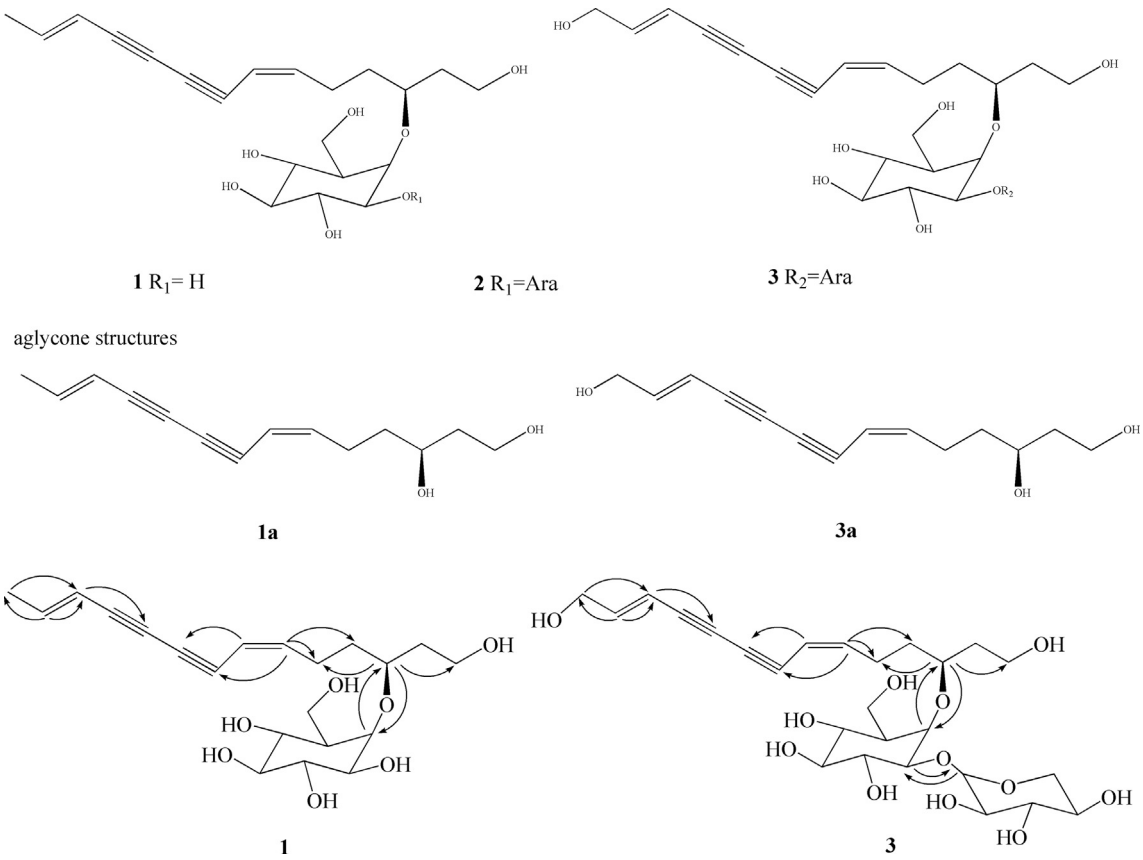

Fig. 1. Compounds isolated from the methanol extract of *C. tinctoria* and key COSY and HMBC correlations for compounds **1** and **3**.

Furthermore, the carbon signal at 17.4 (C-14) in the spectrum of **2** had been replaced by a carbon signal at 61.2 in the spectrum of **3**, indicating that the methyl group at the C-14 position of **2** had been replaced with a hydroxymethyl moiety in **3**. Four olefinic protons in the ^{1}H NMR at δ 6.39 (m, 2H), 5.87 (d, J = 15.76 Hz, 1H), and 5.70 (d, J = 15.68 Hz, 1H) were assigned to *trans* double bonds because of their large coupling constants, suggesting that **3** possesses a (6E, 12E)-tetradecadiene-8,10-diyne-1,3-diol aglycone. The anomeric carbon configuration of the two sugar units were determined as β-glucopyranosyl and β-arabinopyranosyl groups from their $^{3}J_{\text{H-1}', \text{H-2}'}$ and $^{3}J_{\text{H-1}'', \text{H-2}''}$ coupling constants of 7.76 and 6.56 Hz, respectively. The β-glucopyranosyl and β-arabinopyranosyl groups of compound **3** were confirmed as D-glucose and L-arabinose using the same procedures as those used for compounds **1** and **2**. The absolute configuration of C-3 in compound **3** was assigned as *R* by comparison of the $[\alpha]_D$ value with that of reference data. Based on these data and a comparison with data from the literature (Zhang et al., 2013), compound **3** was identified as coreoside B (Fig. 1).

As the main source of polyacetylenes, a large number of polyacetylene glycosides have been isolated from the plants of the Asteraceae family over the last 15 years (Negri, 2015). Most of these compounds have 10, 13 or 15 carbon atoms in their carbon skeleton, namely C_{10}, C_{13}, and C_{15} polyacetylene glycosides (Negri, 2015). It is relatively uncommon to isolate C_{14} polyacetylene glycosides, especially with *cis* double bonds, from plants of the Asteraceae family. Previous studies have shown that plant species of this family can synthesize the same kind of polyacetylenes (Zhang et al., 2013). The results of this study broaden the information of polyacetylene glycosides from plants of the Asteraceae family and may help to identify new polyacetylene glycosides from this family.

2.2. Antimicrobial bioassays

All three compounds were tested for antimicrobial activity against nine pathogenic strains and the MICs values of the compounds are summarized in Table 2. Compound **1** showed high levels of antimicrobial activity — as compared to positive controls, ampicillin and gentamicin — against *Staphylococcus aureus* and *Bacillus anthracis*, with MICs values of 27 ± 0.27 μM and 18 ± 0.40 μM, respectively. Compounds **2** and **3** both showed weak antimicrobial activity against *S. aureus* and *B. anthracis*.

Separation of the methanol extract of *C. tinctoria* buds allowed the isolation of two new and one known polyacetylene glycoside. The structures of these compounds were elucidated using a combination of spectroscopic methods and chemical analysis. To the best of our knowledge, this is the first report concerning isolation of compounds 1 and 2 from *C. tinctoria*.

Table 2
Antimicrobial activity of the compounds isolated from *C. tinctoria.*

Microorganisms	Minimum inhibitory concentration (MIC, μM)				
	1	2	3	Ampicillin	Gentamicin
M. smegmatis	–	–	–	43 ± 0.65	14 ± 0.35
S. aureus	27 ± 0.27	93 ± 0.34	85 ± 0.55	43 ± 0.27	28 ± 0.37
B. subtilis	–	–	–	86 ± 0.28	28 ± 0.78
C. perfringens	–	–	–	22 ± 0.44	14 ± 0.31
M. tetragenus	–	–	–	86 ± 0.46	14 ± 0.61
C. albicans	–	–	–	43 ± 0.35	7 ± 0.28
M. phlei	–	–	–	86 ± 0.48	14 ± 0.19
E.coli	–	–	–	22 ± 0.40	14 ± 0.35
B. anthracis.	18 ± 0.40	73 ± 0.52	68 ± 0.36	43 ± 0.62	14 ± 0.26

Note: The minus (–) means no inhibition was observed.

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B. anthracis is the causative agent of anthrax, which is a highly lethal zoonotic disease found around the world (Wenner and Kenner, 2004). Recently, *B. anthracis* has been used as a bioterrorist agent in the USA and Europe (Hicks et al., 2012; Irenge and Gala, 2012). Although front-line antibiotics have been found that can treat anthrax, *B. anthracis* strains are increasingly becoming resistant (Bouzianas, 2010) and it is crucial to find new antibiotics. All compounds in this study showed antimicrobial activity against *B. anthracis* and among them compound **1** showed potent activity, with the potential to be used as a new antibiotic against *B. anthracis*. The hydrolysates of these polyacetylene glycosides have structural features similar to the parent compounds and may also show antimicrobial activity (Zhang et al., 2013). However, the mechanism of antibacterial activity and the toxicological effects of these compounds remain unknown and require further study.

3. Conclusions

Three polyacetylene glycosides with antimicrobial activity against *B. anthracis* have been isolated from the buds of *C. tinctoria* and chemically identified and characterized. Further studies are needed to elucidate the mechanism of antibacterial activity of these compounds.

4. Experimental

4.1. General experimental procedures

^{1}H, ^{13}C NMR spectra and two-dimensional NMR spectra, including COSY, HSQC and HMBC, were obtained on Bruker Avance 300, 400, or 500 spectrometers (Bruker BioSpin GmbH, Beijing, China), using tetramethylsilane (TMS) as an internal standard. The chemical shifts in the NMR spectra are recorded as δ values. UV spectra were measured on a Beckman DU640 spectrophotometer (Beckman Coulter, Beijing, China). IR spectra were acquired using KBr discs on a Nicolet Nexus 470 spectrophotometer (Thermo Scientific, Beijing, China). Optical rotations were recorded on a Perkin-Elmer 243B digital polarimeter. Electrospray ionization mass spectrometry (ESI-MS) analyses were obtained using on a Q-Tof Ultima Global GAA076 LC mass spectrometer (Waters Asia, Ltd., Singapore). Thin-layer chromatography (TLC) and column chromatography (CC) were performed on plates precoated with silica gel GF254 (10–40 μm), and using silica gel (300–400 mesh, Qingdao Marine Chemical Factory, Qingdao, China), reversed phase C18 (Octadecylsilyl, ODS) silica gel (Silicycle, 50 μm, Parc-Technologique Blvd, Canada) and Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO, USA), respectively.

Dimethyl sulfoxide-d_6 (DMSO-d_6) and MeOH were purchased from Merck (Darmstadt, Germany). All of the other chemicals and solvents used in this study were of analytical grade.

4.2. Plant materials

C. tinctoria was obtained from the Beiyuanchun farmers market in Urumchi city of the Xinjiang Uygur Autonomous Region, China in September 2012. Voucher specimens of *C. tinctoria* were identified by Thomas Nuttall and are deposited in the Key Laboratory of Food Processing and Quality Control, Nanjing Agricultural University, with an index number of XJ8126.

4.3. Extraction and isolation

Fresh buds of *C. tinctoria* (2.5 kg) were dried, homogenized with a Polytron homogenizer and extracted (X 3) with equal volumes of cyclohexane at room temperature to remove oil. Then the residue was extracted with equal volumes of MeOH (X 3) at room temperature. The combined MeOH extraction solutions were filtered and concentrated *in vacuo* at 45 °C to give an extract (32 g).

The latter was separated into three fractions (Fr. 1–3) using silica gel CC (100 g silica gel, 300–400 mesh) by a step-wise gradient elution of petroleum ether:acetone (100:0 to 0:100, v/v). Fractions with antimicrobial activity were subsequently separated by a bioassay-guided method.

Fraction 1 (7.6 g) was separated into two subfractions (Fr. 1-1 and Fr. 1-2) by silica gel CC using $CHCl_3$:MeOH (10:1, v/v) as an eluent. Fr. 1-1 (with antimicrobial activity) was then passed through a silica gel column with a $CHCl_3$:MeOH eluent (5:1, v/v) to yield compound **1** (4.5 mg).

Fraction 2 (9.4 g) was subjected to silica gel CC with a $CHCl_3$:MeOH eluent (1:1, v/v) to obtain two subfractions, Fr. 2-1 and Fr. 2-2 (both with weak antimicrobial activity). Fr. 2-1 was separated by Sephadex LH-20 CC with a $CHCl_3$:MeOH eluent (1:1, v/v) to give compound **2** (3.6 mg). Fr. 2-2 was purified by silica gel CC using a $CHCl_3$:MeOH eluent (5:1, v/v) to obtain compound **3** (9.2 mg).

4.4. Acid hydrolysis of compounds and absolute configuration determination

The method used to determine the absolute configuration was carried out as described (Deyrup et al., 2007) with minor modifications. Briefly, pure compound (2 mg) was individually dissolved in MeOH prior to addition of 1 M HCl (2 mL). Each mixture was heated at 85 °C for 15 h, with the resulting solution extracted with equal volume of EtoAc. Each EtoAc solution was then individually evaporated to remove approximately 90% of the solvent. Then each remaining mixture was individually subjected to silica gel CC using $CHCl_3$ as eluent to give polyacetylene (**1a** obtained from compound **1** and **3a** obtained from compound **3**). Each aqueous layer of the extraction was evaporated *in vacuo* to provide a residue, which was repeatedly distilled to dryness from H_2O *in vacuo* until a neutral residue was obtained. L-Cysteine methyl ester hydrochloride (2 mg in 1 mL of anhydrous pyridine) was next added to each mixture and stirred at 60 °C for 1 h. A mixture of HMDS-TMCS (hexamethyldisilazane: trimethylchlorosilane = 3:1, 300 μL) was also added, with the resulting mixtures stirred individually for another 30 min, with hexane (3 mL) and H_2O (1 mL) then added. The hexane layer was dried (anhyd. Na_2SO_4) before being analyzed by GC-MS. Analysis conditions were as follows: capillary column, HP-5MSi (30 m × 0.25 mm, with a 0.25 μm film, Agilent, USA); detection, FID; injection temperature, 250 °C; initial temperature 160 °C, ramped to 250 °C at 15 °C/min, and held at 250 °C for 10 min.

4.5. Coreoside E (*1*)

Brown amorphous powder. $[\alpha]_D$: −10.2 (MeOH; c = 1.08). UV (MeOH) λ_{max} (ε): 230, 262, 276, 294, and 313 nm. IR (KBr) 3326, 2311 and 1603 cm^{-1}. For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1. HRESI-MS *m/z* 403.1742 $[M + Na]^+$ (calcd. 403.1757 for $C_{20}H_{28}O_7$).

1a. Hydrolysate of compound **1** (Fig. 1). Brown amorphous powder. ^{1}H NMR (300 MHz, MeOH, TMS): δ 6.44 (m, 2H), 5.92 (d, J = 15.92 Hz, 1H), 5.71 (d, J = 16.00 Hz, 1H), 4.20 (m, 2H), 3.74 (m 5H), 2.30 (m, 2H), 2.06 (m, 1H), 1.64 (m, 2H), 1.53 (m, 2H). ^{13}C NMR: δ 17.4, 36.4, 39.6, 59.7, 61.4, 68.7, 72.2, 73.5, 79.3, 80.1, 106.7, 108.2, 147.7, 149.1; ESI-MS *m/z* 241.1 $[M + Na]^+$.

4.6. Coreoside F (*2*)

Brown amorphous powder. $[\alpha]_D$:−7.2 (MeOH; c = 1.15). UV (MeOH) λ_{max} (ε): 230, 262, 276, 294, and 313 nm. IR (KBr) 3425,

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1719, 1653 and 1023 cm^{-1}. For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1. HRESI-MS *m/z* 551.0295 $[M + K]^+$ (calcd. 551.0250 for $C_{25}H_{36}O_{11}$).

4.7. Coreoside B (**3**)

Brown amorphous powder. UV (MeOH) λ_{max} (ε): 239, 247, 278, 294, and 313 nm. ^{1}H NMR (300 MHz, MeOH, TMS): δ 6.39 (m, 2H), 5.87 (d, J = 15.76 Hz, 1H), 5.70 (d, J = 15.68 Hz, 1H), 4.59 (d, J = 6.56 Hz, 1H), 4.50 (d, J = 7.76 Hz, 1H), 4.15 (m, 2H), 4.05 (s, 1H), 3.89 (m, 6H), 3.70 (m, 4H), 3.40 (dd, J = 6.30, 12.63 Hz, 2H), 3.27 (m, 2H), 2.33 (m, 2H), 1.81 (m, 3H), 1.68 (m, 3H). ^{13}C NMR: δ 28.6, 33.7, 36.6, 58.0, 61.2, 65.5, 68.0, 70.0, 71.7, 71.7, 72.7, 73.5, 74.1, 76.2, 76.3, 76.8, 78.3, 79.8, 81.3, 100.9, 104.2, 107.6, 108.2, 145.9, 148.6; HRESI-MS *m/z* 551.1372 $[M + Na]^+$ (calcd. 551.1360 for $C_{25}H_{36}O_{12}$).

3a. Hydrolysate of compound **3** (Fig. 1). Brown amorphous powder. ^{1}H NMR (300 MHz, MeOH, TMS): δ 6.35 (m, 2H), 5.68 (d, J = 15.54 Hz, 1H), 3.65 (m 1H), 2.27 (m, 1H), 1.89 (m, 1H), 1.82 (dd, J = 1.83, 6.84 Hz, 1H), 1.62 (m, 2H), 1.54 (m, 2H). ^{13}C NMR: δ 36.4, 39.6, 59.7, 61.4, 68.7, 72.2, 73.5, 74.1, 79.3, 80.1, 106.7, 108.2, 147.7, 149.1; ESI-MS *m/z* 233.1 $[M - H]^-$.

4.8. Antimicrobial bioassays

Antimicrobial bioassays was carried out individually with each pure compound using the disc diffusion method described by Gomez et al. (1997). Briefly, each agar cup (6 mm diameter) was filled with a 100 μL solution of one of the test compounds dissolved in 5% DMSO at concentrations of 2, 4, 8, 16, 32, 64, 128 and 256 μM. Cups containing DMSO and commercial antimicrobial agents, ampicillin and gentamicin (National Institute for the Control of Pharmaceutical and Biological products, China), were used as negative and positive controls, respectively. The nine test microorganisms used were *Mycobacterium smegmatis* (CMCC 93321), *S. aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Clostridium perfringens* (ATCC 13124), *Micrococcus tetragenus* (ATCC 35098), *Candida albicans* (CMCC 98001), *Mycobacterium phlei* (AS 4.1180), *Escherichia coli* (ATCC 25922) and *B. anthracis* (NX 1.1230). The tested strains were cultivated in Luria–Bertani (LB) agar plates for the bacteria and in YPD agar plates for *C. albicans* at 37 °C. After incubating for 24 h, the zones of inhibition (mm in diameter) were recorded and the minimal inhibitory concentrations (MICs) values determined.

4.9. Statistical analysis

The resulting data are presented as means ± standard deviation in the Supporting information.

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.phytochem.2016.12.023.

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