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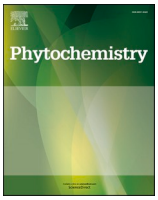

Feruloyl sucrose derivatives from the root of *Xerophyllum tenax*

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ABSTRACT

A phytochemical investigation of the roots of *Xerophyllum tenax* led to the isolation of three undescribed feruloyl sucrose derivatives along with two known feruloyl sucrose derivatives, heloniosides A and B. This is the first report of their occurrence in the genus *Xerophyllum* and the family Melanthiaceae. The structures of these compounds were elucidated on the basis of chemical and spectroscopic analysis including 1D and 2D NMR and analysis of MS-MS fragmentation.

1. Introduction

Xerophyllum tenax (Pursh) Nutt. (Melanthiaceae), commonly known as bear grass, is a perennial characterized by thickened underground rhizomes and tufts of long, slender leaves and flowers crowded on vertical spikes. It is not a grass but a member of the Xerophyllae tribe in the Melanthiaceae family. The family includes the well-known wildflowers in the genera *Trillium* and *Paris*, as well as the genus *Veratrum*, which is rich in steroidal alkaloids (Schep et al., 2012). *Xerophyllum* Michx. comprises two species endemic to North America: *Xerophyllum asphodeloides* (L.) Nutt in the Appalachian Mountains and coastal pine barrens from Alabama to New Jersey, and *X. tenax* in the mountains and foothills of western North America from British Columbia to central California (Utech 2012). Indigenous Americans of the western United States use *X. tenax* for baskets, food, medicine, cosmetics, and for spiritual and ceremonial purposes (Hummel et al., 2012).

Only two phytochemical report on this species is available to date, one focused on the seeds of *X. tenax* and reported the isolation, identification and biological activity of two ecdysteroids (Alison et al., 1997). This was the first report of the occurrence of ecdysteroids in *Xerophyllum* and also in the family Melianthaceae. Another one reported the content of shikimic acid in several plants, including *X. tenax*. (Avula et al., 2009). Given the lack of phytochemical studies on the genus *Xerophyllum*, it remains an interesting target for the discovery of new molecules.

The present study focused on the isolation of feruloyl sucrose derivatives from the roots of *X. tenax*. Ferulic acid derivatives are a type of natural compound composed of a phenylpropanoid nucleus, which are mostly produced by the shikimic acid biosynthetic pathway (Seo et al., 2015). Feruloyl sucrose esters incorporate a central sucrose unit, connected by ester linkages to one or more ferulic acid moieties, and sometimes other ester groups. In the current report, three undescribed feruloyl sucrose derivatives were obtained from the roots of *X. tenax*. Herein we describe the isolation and structure elucidation of these undescribed compounds.

2. Results and discussion

The *n*-BuOH soluble fraction of a methanol extract of the roots of *X. tenax* was fractionated by a combination of chromatographic methods to afford two undescribed feruloyl sucrose esters (**1, 3**) and one undescribed feruloyl sucrose acetate ester (**5**), together with two known compounds (**2, 4**) (Fig. 1). The known compounds were identified as helonioside A (**2**) and helonioside B (**4**) by comparison of 1D and 2D NMR, HR-ESI-TOF-MS with literature data (Fig. S29 – S34 of the Supporting Information) (Nakano et al., 1986; Yan et al., 2008). This is the first time that these compounds have been isolated from the genus *Xerophyllum* and the family Melanthiaceae.

Compound **1** was isolated as a white amorphous powder. The molecular formula was determined to be $C_{32}H_{38}O_{17}$ due to the observation of an $[M + Na]^+$ quasi-molecular ion at *m/z* 717.2013 in the HR-ESI-TOF mass spectrum. All of the individual protons and carbons (Table 1) were assigned by combined analysis of 1D and 2D NMR. The ^{1}H-NMR spectrum (Table 1) of compound **1** showed two typical ABX spin systems of aromatic moieties at δ 7.16 (1H, dd, $J = 8.3$, 2.0 Hz, H-

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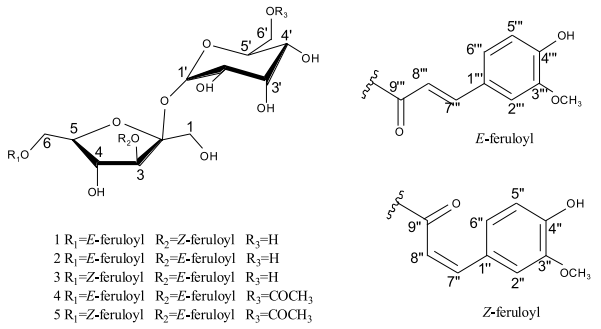

Fig. 1. Chemical structures of **1**–**5**.

6″), δ 6.76 (1H, d, J = 8.3 Hz, H-5″), δ 7.83 (1H, d, J = 2.0 Hz, H-2″), and δ 7.09 (1H, dd, J = 8.3, 2.0 Hz, H-6‴), δ 6.81 (1H, d, J = 8.3 Hz, H-5‴), δ 7.19 (1H, d, J = 2.0 Hz, H-2‴). Together with two pairs of olefinic protons at δ 6.90 (1H, d, J = 12.0 Hz, H-7″), 5.89 (1H, d, J = 12.0 Hz, H-8″) and 7.65 (1H, d, J = 16.0 Hz, H-7‴), 6.39 (1H, d, J = 16.0 Hz, H-8‴), they indicated the presence of two feruloyl groups in compound **1**. As the coupling constant was 16.0 Hz for protons at δ 7.65/6.39 and 12.0 Hz for protons at δ 6.90/5.89, one of these was an (*E*)-feruloyl, while the other was a (*Z*)-feruloyl group (Kim et al., 2010). The position of the two methoxy groups in feruloyl units was assigned on the basis of NOESY correlations, which showed that the correlation between two methoxy groups and aromatic protons: δ 3.87 with H-2'' ; and δ 3.89 with H-2‴. Moreover, eight oxygenated methines at δ 5.47 (d, J = 8.0 Hz, H-3), 4.40 (t, J = 8.0 Hz, H-4), 4.13 (m, H-5), 5.43 (d, J = 4.0 Hz, H-1′), 3.41 (dd, J = 10.0, 4.0 Hz, H-2′), 3.62 (t, J = 9.5 Hz, H-3′), 3.39 (t, J = 9.5 Hz, H-4′), and 3.88 (m, H-5′), three oxygenated methylenes at δ 3.60 and 3.65 (each 1H, d, J = 12.0 Hz, H-1), 4.46 (dd, J = 11.9, 3.6 Hz, H-6a), 4.54 (dd, J = 11.9, 7.3 Hz, H-6b), 3.79 (dd, J = 12.0, 4.5 Hz, H-6′a) and 3.87 (dd, J = 12.0, 2.5 Hz, H-6′b) were observed in the ^{1}H-NMR spectrum, indicating the presence of two sugars. A characteristic anomeric proton signal at δ 5.43 with a small coupling constant (d, J = 4.0 Hz, H-l'), two anomeric carbons at δ 105.0 (C-2) and 93.1 (C-1′), together with another 10 oxygenated carbon signals at δ 65.2 (C-1), 79.0 (C-3), 74.8 (C-4), 81.2 (C-5), 66.1 (C-6), 73.2 (C-2′), 74.9 (C-3′), 71.3 (C-4′), 74.4 (C-5′) and 65.2 (C-6′) observed in the ^{13}C-NMR spectrum, established the sugar to be a sucrose moiety (Cho et al., 2015). The presence of sucrose was further confirmed by alkaline and acid hydrolysis, and comparison of the TLC behavior and optical rotations of the resulting sugars with standard samples. The HMBC correlation from H-3 (δ 5.47) to C-9" (δ 167.3) confirmed that the (*Z*)-feruloyl group was located at C-3 of the fructose moiety, while the correlations from H-6a (δ 4.46) and H-6b (δ 4.54) to C-9‴ (δ 169.1) confirmed that the (*E*)-feruloyl group was located at C-6 of the fructose moiety (Fig. 2). Therefore, compound **1** was identified as 3-(*Z*)-feruloyl-6-(*E*)-feruloyl sucrose (Fig. 1). This is the first report of both an *E*-feruloyl group and a *Z*-feruloyl group occurring together in one feruloyl sucrose ester.

It was noted that over time the ^{1}H-NMR spectra of both compounds 1 and 2 were subject to change (Fig. 3). For compound **1**, over a period of several months, characteristic proton signals corresponding to the presence of the *Z*-double bond lowered in intensity, and new signals corresponding to the presence of an *E*-double bond appeared, suggesting that compounds **1** was gradually converting into compound **2**. Based on their integrals in the ^{1}H-NMR spectra, the percentage of compounds **1**

Table 1
^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data for compounds 1–3 in CD_3OD.

	1		3		5	
Position	H	C	H	C	H	C
β-D-Fru 1a	3.60 (1H, d, 12.0)	65.2	3.57 (1H, d, 12.0)	65.1	3.56 (1H, d, 12.0)	65.4
1b	3.65 (1H, d, 12.0)		3.60 (1H, d, 12.0)		3.60 (1H, d, 12.0)	
2		105.0		105.2		105.0
3	5.47 (1H, d, 8.0)	79.0	5.46 (1H, d, 7.5)	79.4	5.47 (1H, d, 8.0)	79.1
4	4.40 (1H, t, 8.0)	74.8	4.38 (1H, t, 7.5)	75.3	4.43 (1H, t, 8.0)	74.8
5	4.13 (1H, m)	81.2	4.14 (1H, m)	81.4	4.14 (1H, m)	81.3
6a	4.46 (1H, dd, 11.9, 3.6)	66.1	4.45 (1H, dd, 11.9, 3.7)	66.1	4.47 (1H, dd, 11.9, 3.7)	65.9
6b	4.54 (1H, dd, 11.9, 7.3)		4.52 (1H, dd, 11.9, 7.5)		4.51 (1H, dd, 11.9, 7.3)	
α-D-Glu 1′	5.43 (1H, d, 4.0)	93.1	5.40 (1H, d, 3.8)	93.3	5.42 (1H, d, 3.8)	92.7
2′	3.41 (1H, dd, 10.0, 4.0)	73.2	3.42 (1H, dd, 9.8, 3.8)	73.2	3.43 (1H, dd, 9.7, 3.8)	73.0
3′	3.62 (1H, t, 9.5)	74.9	3.67 (1H, t, 9.5)	74.9	3.63 (1H, t, 9.5)	74.9
4′	3.39 (1H, t, 9.5)	71.3	3.39 (1H, t, 9.5)	71.3	3.25 (1H, t, 9.5)	71.9
5′	3.88 (1H, m)	74.4	3.94 (1H, m)	74.4	4.17 (1H, m)	72.2
6′a	3.79 (1H, dd, 12.0, 4.5)	65.2	3.77 (1H, dd, 12.0, 4.5)	62.7	4.10 (1H, dd, 11.9, 7.2)	65.8
6′b	3.87 (1H, dd, 12.0, 2.5)		3.90 (1H, dd, 12.0, 2.6)		4.57 (1H, dd, 11.9, 1.2)	
3-*Z*-Feruloyl 1″		128.0	**6-*Z*-Feruloyl**	128.0	**6-*Z*-Feruloyl**	128.1
2″	7.83 (1H, d, 2.0)	115.2	7.82 (1H, d, 2.0)	115.2	7.84 (1H, d, 2.0)	115.1
3″		148.3		148.3		148.3
4″		149.7		149.4		149.6
5″	6.76 (1H, d, 8.3)	115.7	6.76 (1H, d, 8.3)	115.7	6.76 (1H, d, 8.2)	115.7
6″	7.16 (1H, dd, 8.3, 2.0)	127.1	7.13 (1H, dt, 8.3, 2.0)	127.0	7.12 (1H, dt, 8.2, 2.0)	126.9
7″	6.90 (1H, d, 12.0)	146.7	6.88 (1H, d, 12.5)	146.5	6.86 (1H, d, 12.8)	145.9
8″	5.89 (1H, d, 12.0)	115.8	5.82 (1H, d, 12.5)	116.0	5.82 (1H, d, 12.8)	116.1
9″		167.3		168.0		167.9
OCH_3	3.87 (1H, s)	56.4	3.87 (1H, s)	56.5	3.86 (1H, s)	56.5
6-*E*-Feruloyl 1‴		127.7	**3-*E*-Feruloyl**	127.7	**3-*E*-Feruloyl**	127.7
2‴	7.19 (1H, d, 2.0)	111.7	7.22 (1H, d, 2.0)	112.1	7.24 (1H, d, 2.0)	112.1
3‴		149.4		149.7		149.4
4‴		150.7		150.8		150.7
5‴	6.81 (1H, d, 8.3)	116.5	6.80 (1H, d, 8.3)	116.5	6.80 (1H, d, 8.2)	116.5
6‴	7.09 (1H, dd, 8.3, 2.0)	124.3	7.13 (1H, dt, 8.3, 2.0)	124.2	7.12 (1H, dt, 8.2, 2.0)	124.3
7‴	7.65 (1H, d, 16.0)	147.2	7.70 (1H, d, 16.0)	147.8	7.70 (1H, d, 16.0)	147.9
8‴	6.39 (1H, d, 16.0)	115.2	6.42 (1H, d, 16.0)	115.0	6.43 (1H, d, 16.0)	114.9
9‴		169.1		168.2		168.2
OCH_3	3.89 (1H, s)	56.5	3.89 (1H, s)	56.4	3.89 (1H, s)	56.4
OAc-6′					2.10 (1H, s)	173.0
						20.9

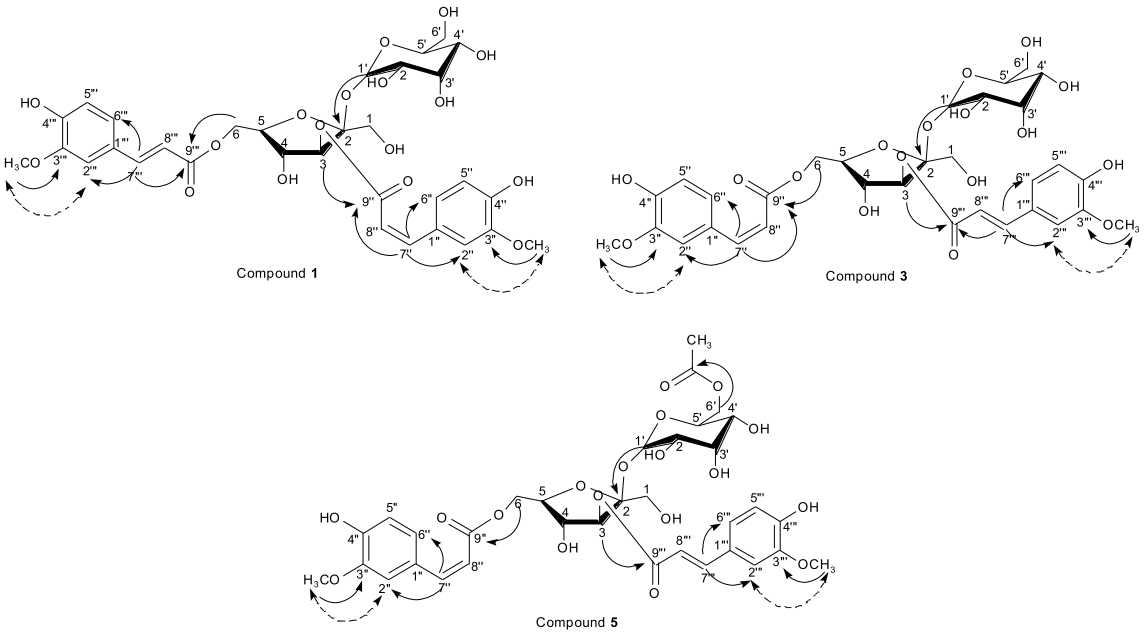

Fig. 2. Selected HMBC (solid arrows) and NOESY (dashed arrows) correlations for compounds **1**, **3** and **5**.

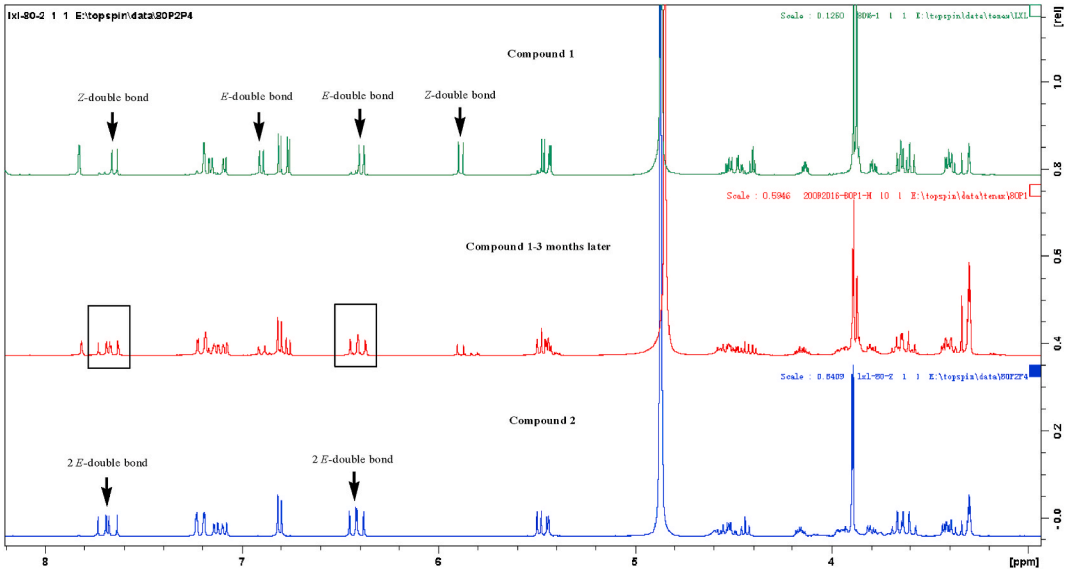

Fig. 3. Isomerization of double bond in compound **1**, as shown by change in ^{1}H-NMR over time (CD_3OD).

and **2** was 37% and 63%, respectively. Ferruloyl derivatives have been shown previously to isomerize from *Z* to *E*, in particular when under UV illumination (Hwang et al., 2016), however in this instance isomerization occurred despite the sample being stored in the dark.

Compound **3** was obtained as a white amorphous powder. Based on a $[M+Na]^+$ quasi-molecular ion at *m/z* 717.2012 in the HR-ESI-TOF-MS spectrum, the molecular formula was determined to be $C_{32}H_{38}O_{17}$, indicating that it was an isomer of compound **1**. Comparing the ^{1}H and ^{13}C NMR spectra of compounds 1 and 3 (Table 1) suggested that compound **3** was also composed of two feruloyl units and one sucrose. The coupling constant of 16.0 Hz for the protons at δ 7.70/6.42 and that of 12.5 Hz for the protons at δ 6.88/5.82 again showed the presence of both (*E*)-feruloyl and (*Z*)-feruloyl groups. From the HBMC spectrum, correlations from H-3 (δ 5.46) to C-9‴ (δ 168.2) confirmed that, in contrast to compound **1**, in compound **3** the (*E*)-feruloyl group is located at C-3 of the fructose moiety, while correlations from H-6a (δ 4.45) and H-6b (δ 4.52) to C-9" (δ 168.0) confirmed that the (*Z*)-feruloyl is located at C-6 of the fructose moiety. Therefore, compound **3** was identified as 3-(*E*)-feruloyl-6-(*Z*)-feruloyl sucrose (Fig. 1).

Compound **5** was isolated as a yellowish gum. The molecular formula was determined to be $C_{34}H_{40}O_{18}$ from a $[M + Na]^+$ quasi-molecular ion at *m/z* 759.2126 in the HR-ESI-TOF mass spectrum. On the basis of the ^{1}H and ^{13}C NMR spectra data (Table 1), compounds **5** and **3** were almost identical, except that compound **5** had additional signals corresponding to the presence of an acetate group. Combined with the coupling constants of 16.0 Hz for the protons at δ 7.70/6.43 and of 12.8 Hz for the protons at δ 6.86/5.82, this indicated that compound **5** possessed a structure containing one sucrose unit, one (*Z*)-feruloyl moiety, one (*E*)-feruloyl moiety and one additional acetate group. Correlations of the oxygenated methylene proton signals H-6′a (δ 4.10) and H-6′b (δ 4.57) with the acetate carbonyl resonance at δ 173.0 indicated that the acetyl group was located at C-6′ of the glucose moiety. In addition, correlation

of the proton signal H-3 (δ 5.47) with the carbonyl carbon signal C-9‴ (δ 168.2) indicated that the (*E*)-feruloyl group is located at C-3 of the fructose moiety, and correlation of the proton signal H-6 (δ 4.51/4.47) with the carbonyl carbon signal C-9" (δ 167.9) indicated that the (*Z*)-feruloyl group is located at C-6 of the fructose moiety (Fig. 2). Overall, the structure of **5** was determined to be 6′-acetyl-3-(*E*)-feruloyl-6-(*Z*)-feruloyl sucrose (Fig. 1).

The presence of the undescribed feruloyl sucrose derivatives, together with helonioside A and helonioside B, constitutes the first report of this compound class in the family Melanthiaceae. Compounds **1**, **2** and **3** are isomers, as are compounds **4** and **5**. According to HRESI-LC-MS^2 analysis, in both positive and negative mode, compounds **1**–**5** share a similar fragmentation pathway (Fig. S27 and S28 of the Supporting Information). Based on the results of MS^2 analysis, a fragmentation pathway was proposed (Fig. 4). Fig. 4A shows the MS-MS spectrum of compound **1** under positive mode, which revealed an $[M + NH_4]^+$ adduct ion at *m/z* 712.2455 and major fragment ions at *m/z* 515.1553, 303.0864, 177.0548, 145.0286, and 117.0339. The cleaving of the glycosidic bond in MS-MS is a common feature of feruloyl sucrose derivatives, along with the loss of other side chains (Wu et al., 2010). The ion at *m/z* 515.1553 was consistent with the elimination of one glucose unit, which could be followed by loss of a ferulic acid and H_2O to yield a product ion at *m/z* 303.0864. A product ion at *m/z* 177.0548 was consistent with the presence of ferulic acid: smaller fragment ions at *m/z* 145.0286 and 117.0339 are also consistent with previous reports (Fig. 4C) (Asamenew et al., 2019; Qi et al., 2008). Compounds **2**–**5** showed almost identical MS-MS spectra to **1**.

In negative mode, alternative MS^2 fragmentation patterns were observed (Fig. 4B). The parent ion at *m/z* 693.2052 for compound **1** can lose a ferulic acid unit to give an ion at *m/z* 517.1574, which then eliminates one molecule of glucose to yield a product ion at *m/z* 337.0939. The ion at *m/z* 175.0404 is consistent with a ferulic acid fragment which can fragment further to give the ion at *m/z* 160.0169 indicating the loss of a further methyl group. (Fig. 4D). Unlike in positive mode, in negative mode compounds **4** and **5** showed a different fragmentation pattern from **1**–**3**, with several of the higher-mass fragments shifted 42 amu higher, consistent with the presence of an additional acetyl group. However, in neither positive nor negative mode were there any significant differences between the MS-MS spectra of the various *cis-trans* isomers: MS-MS analysis alone cannot be used to distinguish these compounds.

Compounds **1**–**5** were tested for antimicrobial activity against a small suite of test microbes, but did not show any activity. Several studies have already shown that feruloyl sucrose derivatives possess multiple types of pharmacological activity, including antioxidant (Choi et al., 2012), cytotoxic (Yan et al., 2008; Kiem et al., 2008), anti-inflammatory (Zheng et al., 2012), and tyrosinase inhibitory activities (Panda et al., 2011; Tanimoto et al., 2006). Thus, the

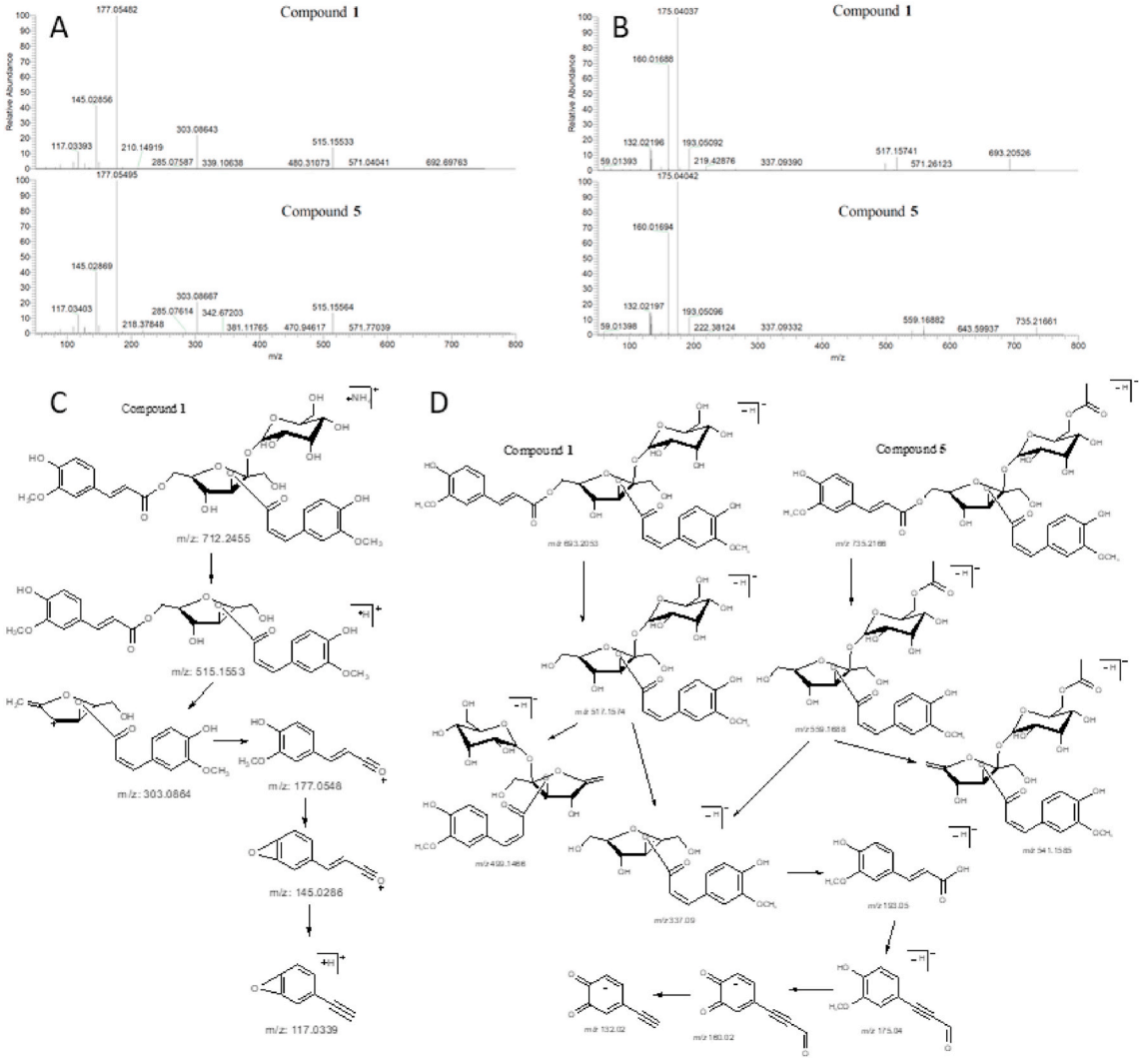

Fig. 4. Proposed fragmentation pathway of compound **1**–**5**. A. The MS^2 spectrum of compounds **1** and **5**; positive mode. B. The MS^2 spectrum of compound **1** and **5**; negative mode. C. Proposed fragmentation pathway of compound **1**; positive mode. D. Proposed fragmentation pathway of compounds **1** and **5**; negative mode.

undescribed feruloyl sucrose derivatives may possess similar biological properties, which need to be explored further.

The botanical classification of *X. tenax* has been changed in the past, being assigned to both the Melanthiaceae (Brummitt, 1992) and Liliaceae (Cronquist, 1981). In 2000, it was assigned to the family Melanthiaceae according to molecular phylogenetic analysis (Rudall et al., 2000; Vance et al., 2004). While our study has established that feruloyl sucrose derivatives are a part of the chemical composition of *X. tenax*, previous studies have shown that feruloyl sucrose derivatives also appear in Liliaceae (Nakano et al., 1986; Satou et al., 1996; Chen et al., 2000; Shu et al., 2002; Ono et al., 2007; Yan et al., 2008; Sun et al., 2012; Munafo and Gianfagna, 2015; Luo et al., 2018). This is consistent with the close relationship between the Liliaceae and Melanthiaceae families.

3. Experimental

3.1. General

The melting points were measured on a Micro-melting point apparatus with corrections. Optical rotations were recorded on an AUTOPOL II Polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). UV spectra were measured using a Hitachi U3900 spectrophotometer (Hitachi, Kyoto, Japan). The high-resolution electrospray ionization mass spectra (HR-ESI-MS) were measured using a MicroTOF-QII spectrometer (Bruker Daltonics, Bremen, Germany) or a Q Exactive HF Orbitrap LC-MS (Thermo Fisher Scientific, Waltham MA, USA). NMR spectra were recorded on an Avance III 600 MHz spectrometer (Bruker BioSpin, Billerica MA, USA) using TMS or the residual solvents as internal standard. For column chromatography, Diaion HP-20 (Mitsubishi Chemical Industries, Tokyo, Japan) was used. Medium pressure liquid chromatography (MPLC) was performed using an ISCO CombiFlash Rf + instrument (Teledyne ISCO, Lincoln NE, USA) equipped with prepacked spherical ODS silica gel (20–45 μm) column (SepaFlash, Santai Technologies, Changzhou, China). Analytical and semipreparative HPLC separations were performed on an Agilent 1260VL quad gradient system (G1311C pump, G1329B autosampler, G1316A thermostatted column compartment and G1315D photodiode array detector, Agilent Technologies, Santa Clara CA, USA), using Hypersil Gold C18 column (3 μm, 2.1 × 150 mm, Thermo Scientific, Waltham MA, USA). Preparative HPLC separations were performed on an Agilent 1260VL quad gradient system (G1311C pump, and G1315D photodiode array detector) equipped with a Rheodyne 7725i manual injection valve (IDEX Health & Science, Middleboro MA, USA) and Shimadzu CTO-20A column oven (Shimadzu Scientific Instruments, Kyoto, Japan). Preparative separations were conducted using a Hypersil Gold C18 column (5 μm, 21.2 × 250 mm). All solvents used were of HPLC grade (Concord Technologies, Tianjin, China). Thin-layer chromatography (TLC) was carried out on silica gel GF_{254} plates (Haiyang Chemicals Corp., Qingdao, China). TLC spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH (v/v).

3.2. Plant material

The roots of *Xerophyllum tenax* (Pursh) Nutt. (Melanthiaceae) were collected in Plumas County, CA, USA (lat 39.8283, lon −121.304, 1625 m elev) on 20 Aug 2003. Specimens were authenticated by Daniel Atha of the New York Botanical Garden. Voucher specimens (Lowell Ahart 10520) have been deposited in the herbarium of the New York Botanical Garden (CHSC, NY-199257). Plant material was air dried in the shade, freed of extraneous material and ground to a coarse powder prior to extraction.

3.3. Extraction and isolation

A one kg dried sample of the root part of *X. tenax* was extracted with 3 × 4 L of methanol at ambient temperature (24 h each time). The pooled methanol extracts were evaporated to dryness *in vacuo* to afford a tarry residue. Then, the methanolic extract (28.0 g) was dispersed in methanol:water (9:1, 100 ml) and defatted with *n*-hexane (3 × 100 ml). The hydroalcoholic phase was freed of solvent, dispersed in water and successively extracted with dichloromethane (DCM) and *n*-BuOH (each 3 × 100 ml) to afford *n*-hexane (1 g), DCM (2.5 g), *n*-BuOH (6.0 g) and aqueous (12.5 g) fractions.

The *n*-BuOH fraction was subjected to column chromatography on Diaion HP-20 (150 g) eluting with a H_2O–MeOH step gradient (each 3 × 200 ml) to give sub-fractions H_2O (1.2 g), 20% MeOH–H_2O (0.4 g), 40% MeOH–H_2O (0.15 g), 60% MeOH–H_2O (0.2 g), 80% MeOH–H_2O (2.5 g), 100% MeOH–H_2O (0.9 g) successively.

The 80% MeOH–H_2O fraction (2.5 g) was separated using flash chromatography (Spherical C18, 20–45 μm, 80 g) with a H_2O-ACN gradient solvent system (90:10 → 0:100, v/v, 81.9 min, 25 ml/min) to give fractions 1, 2, 3 and 4. Fractions 1–3 were separated on preparative RP-18 column (5 μm, 21.2 × 250 mm, 40 °C, 10 ml/min) using H_2O-ACN (80:20, v/v) and afforded compound **1** (7.5 mg), compound **2** (20.5 mg), and compound **3** (6.0 mg). Fraction 4 was separated following the same procedure and afforded compound **4** (16.3 mg) and compound **5** (6.4 mg). HPLC retention times were 4.27 min, 4.70 min, 5.10 min, 7.59 min and 8.39 min, respectively (Hypersil Gold C18, 25% MeCN:H_2O containing 0.1% formic acid under 0.2 ml/min, 40 °C).

3.3.1. Compound *1*

White, amorphous powder; mp 136–138 °C; $[\alpha]_D^{25}$ -10.3 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.88), 239 (3.85), 299 (3.94), 328 (4.12); ^{1}H-NMR (CD_3OD, 600 MHz), see Table 1; ^{13}C-NMR (CD_3OD, 150 MHz), see Table 1; HRESIMS *m/z* 717.2013 $[M + Na]^+$ (calcd. for $C_{32}H_{38}O_{17}Na$, 717.2001).

3.3.2. Compound *3*

White, amorphous powder; mp 136–138 °C; $[\alpha]_D^{25}$ -7.3 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.88), 239 (3.85), 299 (3.94), 328 (4.12); ^{1}H-NMR (CD_3OD, 600 MHz), see Table 1; ^{13}C-NMR (CD_3OD, 150 MHz), see Table 1; HRESIMS *m/z* 717.2012 $[M + Na]^+$ (calcd. for $C_{32}H_{38}O_{17}Na$, 717.2001).

3.3.3. Compound *5*

Yellowish gum; mp 179–181 °C; $[\alpha]_D^{25}$ -10.3 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.90), 239 (3.88), 299 (3.97), 328 (4.15); ^{1}H-NMR (CD_3OD, 600 MHz), see Table 1; ^{13}C-NMR (CD_3OD, 150 MHz), see Table 1; HRESIMS *m/z* 759.2126 $[M + Na]^+$ (calcd. for $C_{34}H_{40}O_{18}Na$, 759.2107).

3.3.4. Alkaline hydrolysis of *1*, *3* and *5*

Compounds (2 mg, each, individually) were dissolved in 3% KOH–MeOH (2 mL), after 6 h, the reaction solutions were neutralized with 1N HCl and then extracted with $CHCl_3$. From the H_2O layer, sucrose was identified by comparing its TLC behavior with standard samples [Si gel, developed with $CHCl_3$–MeOH–H_2O (6:4:1)] (Shu et al., 2002).

3.3.5. Acid hydrolysis of *1*, *3* and *5*

A solution of **1**, **3** and **5** (2 mg each, individually) in 3% HCl (2 mL) was heated for 4 h. Then, the solution was evaporated *in vacuo* and the residues were subjected to TLC analysis. The neutralization solutions were centrifuged, filtrated and furtherly isolated on a LC-2030 C Prominence-I system (Shimadzu, Kyoto, Japan) equipped with a RID-20 A refractive index detector, using a Waters XBridge BEH Amide column (10 × 250 mm, 5 μm). The flow rate was 3 mL/min, and the mobile phase was acetonitrile-water (75:25). D-glucose and D-fructose were confirmed by compared optical rotation with authentic samples: D-glucose, [α]25 D +52.5 (*c* 0.05, H_2O); D-fructose, $[\alpha]_D^{25}$ -67 (*c* 0.05, H_2O) (Zhao et al., 2019), and by comparing their TLC behavior with standard

samples [Si gel, developed with $CHCl_3$–MeOH–H_2O (6:4:1)] (Shu et al., 2002).

3.3.6. LC-MS/MS analysis

A Q Exactive HF orbitrap mass spectrometer coupled with an Ultimate3000 RSLC nano (Thermo Fisher Scientific, USA) was used for LC-MS/MS analysis in both positive and negative ion modes. An ODS column (Pursuit XRs C18, 4.6 mm × 150 mm, Agilent, USA) was used for LC separation. The mobile phase A was 100% acetonitrile with 0.1% formic acid and the mobile phase B was water with 0.1% formic acid. The separation was performed as follows, 70% (B) in 25 min and then up to 100 (B) from 25 min to 35 min. Mass resolutions of 120,000 and 30,000 were applied for precursors and fragments. Data dependent MS/MS acquisition with fragmentation of the top five most intense precursors was performed in the analysis. MS/MS spectra were acquired with stepped normalized collision energy (NCE) of 20, 40 and 60. The other detailed ion source parameters are as follows: spray voltage, 3.8 kV for positive (and 3.0 kV for negative); capillary temperature, 250 °C; sheath gas flow rate (arb), 45; aux gas flow rate (arb), 12; probe heater temperature, 300 °C; mass range (m/z), 100–1500.

3.3.7. Antimicrobial testing

Antimicrobial assays were carried out according to literature protocols (Li and Clark, 2020) against the following test microbes: *Staphylococcus epidermidis* (ATCC 14990), *Pseudomonas aeruginosa* (ATCC 15692), *Pseudomonas fluorescens* (ATCC 13525) *Bacillus subtilis* (ATCC 6633) and *Candida albicans* (ATCC 76615).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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