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•Research article•

Lignans with (*N*, *N*-diethyl)methyl amino group from *Buxus rugulosa*

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[ABSTRACT] Buxrugulosides A–E, four lignan glycosides (**1–4**) and a protocatechuate derivative (**5**) featuring a rare (*N*, *N*-diethyl)methyl amino group at aromatic rings, were obtained from the aerial parts of *Buxus rugulosa,* which is famous for treating coronary heart disease. Their structures including absolute configurations were elucidated by HRMS, 1D and 2D NMR, and by comparing their CD data with previous reports. Compound **1** was a rare sesquilignan, and all of these compounds were the first example of lignans with (*N*, *N*-diethyl)methyl amino group.

[KEY WORDS] Lignan glycoside; (*N*, *N*-diethyl)methyl amino group; *Buxus rugulosa*
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Introduction

The genus *Buxus* belongs to the family Buxaceae, which is extensively distributed in the Middle East, tropical and South Africa, East Asia, and North and Central America [1]. In addition to being widely planted to beautify the environment as evergreen shrubs, plants of the genus *Buxus* have also been used in traditional medicine for the treatment of fatigue, rheumatism, malaria, depression, and skin infection [2]. Steroidal alkaloids, as the major bioactive components, were found to possess potent cardio-protective, anti-tumor, antiacholinesterase, antifungal and immunosuppressive activities and have been isolated in large amounts from this genus, while a few other chemical constituents were reported [1-5]. *Buxus rugulosa* is found in the northwest Yunnan Province of China. To date, eight new steroidal alkaloids have been isolated from this plant [3-4]. To continue searching for bioactive agents from this plant, we investigated the aerial parts of *B. rugulosa* and consequently isolated four lignan glycosides and a protocatechuate derivative with a rare (*N*, *N*-diethyl)methyl amino group at aromatic rings, named buxrugulosides A–E (**1–5**) (Fig. 1). Herein, we described the isolation and structural elucidation of these compounds.

Results and Discussion

Compound **1** was isolated as yellow gum. The HR-ESIMS exhibited a quasi-molecular ion peak at *m*/*z* 832.3748 ($[M + H]^+$, Calcd. 832.3750), indicating the molecular formula $C_{42}H_{57}NO_{16}$. The 1D and 2D NMR data of **1** (Table 1) indicated the presence of one 1, 3, 4, 5-tetrasubstituted benzene ring [δ_H 6.88 (1H, br s, H-2′) and 6.69 (1H, s, H-6′)], one 1, 3, 4-trisubstituted benzene moiety [δ_H 6.87 (1H, overlap, H-6″), 7.07 (1H, d, J = 8.3 Hz, H-5″) and 7.02 (1H, d, J = 1.5 Hz, H-2″)], one symmetrical 1, 3, 4, 5-tetrasubstituted benzene ring where two equivalent aromatic protons resonated at δ_H 6.64 (2H, s, H-2, 6) and a 2, 6-diaryltetra-hydrofuran ring system [6], possessing the characteristic signals [δ_C 87.5 (C-7′), 87.3 (C-7), 72.9 (C-9), 72.8 (C-9′), 55.8 (C-8′), 55.3 (C-8)]. In addition, the resonances attributable to a glucosyl moiety and a propan-1, 2, 3-triol moiety were observed in the NMR spectra. Detailed analysis of the above data of **1** suggested that compound **1** was a sesquilignan monoglucoside [7]. According to HMBC spectrum (Fig. 2), four aromatic methoxy signals [δ_H 3.81 (3H, overlap), 3.83 (3H, s) and 3.80 (6H, s)] were attached to C-3′, C-4′, C-3 and C-5, respectively. The multiplicity of anomeric proton [δ_H 4.85 (d, J = 7.7 Hz)] and ^{13}C NMR chemical shifts of monosaccharide [δ_C 102.7 (C-1‴), 74.9 (C-2‴), 78.2 (C-3‴), 71.4 (C-4‴), 77.8 (C-5‴), 65.7 (C-6‴) indicated the typical signals of a β-D-glucopyranosyl moiety [8]. The glucose

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These authors have no conflict of interest to declare.
Dedicated to the 90th Anniversary of the Founding of Shenyang Pharmaceutical University

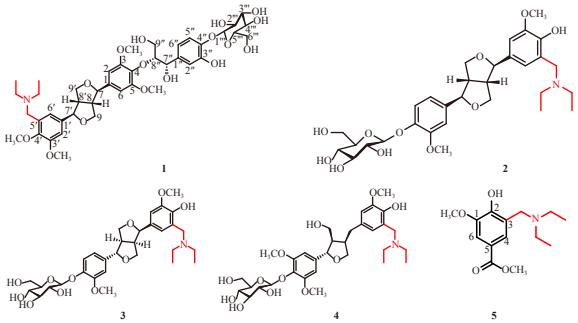

Fig. 1 Structures of compounds 1–5

Table 1 NMR Data of 1 [a] in CD_3OD (δ in ppm, *J* in Hz)

Position	δ_C	δ_H	Position	δ_C	δ_H
1	138.9		5″	117.1	7.07, d (8.3)
2	104.2	6.64, s	6″	120.9	6.87 [b]
3	154.4		7″	73.8	4.90, d (5.7)
4	136.0		8″	86.9	4.27 [b]
5	154.4		9″	62.6	3.87 [b]; 3.59, dd (3.5, 12.0)
6	104.2	6.64, s	3-OCH_3	56.5	3.80, s
7	87.3	4.72, d (4.1)	5-OCH_3	56.5	3.80, s
8	55.3	3.12 [b]	3′-OCH_3	56.7	3.81, s
9	72.9	4.25 [b]; 3.86 [b]	4′-OCH_3	55.8	3.83, s
1′	132.6		1‴	102.7	4.85, d (7.7)
2′	110.4	6.88 br s	2‴	74.9	3.44 [b], m
3′	148.4		3‴	78.2	3.37, m
4′	149.3		4‴	71.4	3.35, m
5′	123.3		5‴	77.8	3.43 [b], m
6′	119.9	6.69, s	6‴	65.7	3.66, dd (5.4, 12.1); 3.86 [b]
7′	87.5	4.67, d (4.3)	5′-$CH_2N(CH_2CH_3)_2$		
8′	55.8	3.11 [b]		57.1	3.81 [b]
9′	72.8	4.25 [b]; 3.86 [b]		47.5	2.65 [b], q (7.1)
1″	137.3			47.5	2.65 [b], q (7.1)
2″	112.1	7.02, d (1.5)		11.5	1.09 [b], t (7.2)
3″	150.3			11.5	1.09 [b], t (7.2)
4″	147.2				

[a] Recorded at 600 MHz for ^{1}H NMR and 151 MHz for ^{13}C NMR; [b] Signals overlapped

group was assigned at C-4″ position by the HMBC correlation from H-1‴ to C-4″. The correlation of H-8″ with C-4 indicated that phenylpropanoid glycoside group was connected to C-4 *via* an ether bridge. Furthermore, an additional set of signals [(δ_H 3.81 (2H, s), 2.65 (4H, q, *J* = 7.1 Hz), 1.09 (6H, t, *J* = 7.2 Hz); δ_C 57.1, 47.5, 11.5] assigned to an (*N*, *N*-diethyl)methyl amino moiety were observed. The (*N*, *N*-diethyl)methyl amino moiety was located at C-5′ by the HMBC correlations from the protons (δ_H 3.81) of the tertiary amine methylene to C-4′ and C-6′. The relative configuration of **1** was mainly determined by the chemical shift differences of H_2-9 and H_2-9′ (Δδ H-9 and Δδ H-9′) and ROESY spectra. The relatively small coupling constant of H-7″ signal at δ_H 4.90 (1H, d, *J* = 5.7 Hz) indicated that the glycerol moiety was in *erythro*-configuration [9]. Both Δδ H-9 and Δδ H-9′ had medium values (0.39), suggesting 7-H/8-H *trans* and 7′-H/8′-H *trans* [10]. The ROESY correlations between H-8 (δ_H 3.12) and H-9*β* (δ_H 4.25), H-7 (δ_H 4.72) and H-9*α* (δ_H 3.86) further confirmed the postulated arrangement. The negative Cotton effect at 238 nm in CD spectra indicated that **1** possessed an 8″*R* configuration [9]. Furthermore, the CD data were consistent with those of terminaloside H [11]. Based on the above analysis, the absolute configuration of **1** was assigned as to be 7*R*, 8*S*, 7′*R*, 8′*S*, 8″*R*, 7″*S*. Thus, the structure of **1** was determined as shown and named buxruguloside A.

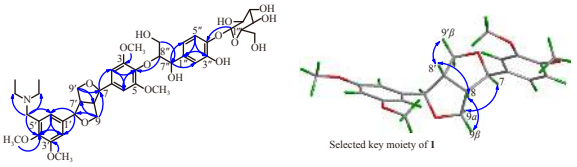

Fig. 2 Key HMBC (H → C) and ROESY (↔) correlations of 1

Compound **2** was isolated as yellow gum. The HR-ESI-MS exhibited a quasi-molecular ion peak at *m*/*z* 606.2904 ([M + H]$^+$, Calcd. 606.2909), indicating the molecular formula $C_{31}H_{43}NO_{11}$. Comprehensively comparing the NMR data (Table 2) of **2** with those of **1** displayed that compound **2** was a lignan glycoside instead of a sesquilignan glycoside. The large coupling constant of the anomeric proton (δ_H 4.81 d, *J* = 7.3 Hz) demonstrated that the glucose was *β*-anomeric configuration [12]. The cross-peak of H-1″ with C-4′ (δ_C 145.7) suggested glucosyl moiety was linked to C-4′. The HMBC correlations from CH_3O- (δ_H 3.82) to C-3 (δ_C 148.1) and from CH_3O- (δ_H 3.70) to C-3′ (δ_C 149.2) indicated that the methoxy groups were bounded to C-3 and C-3′, respectively. Similar with **1**, compound **2** also possessed an (*N*, *N*-diethyl)methyl amino moiety, which was located at C-5 by the HMBC correlations of tertiary amine methylene protons at δ_H 3.71 with C-4 (δ_C 147.5) and C-6 (δ_C 118.2). The relative configuration of **2** was determined by the values of Δδ H-9 and Δδ H-9′. The calculation results showed that both Δδ H-9 and Δδ H-9′ have medium values (0.39), suggesting 7-H/8-H *trans* and 7′-H/8′-H *trans* [10]. This deduction was consistent with the ROESY correlations of H-8 (δ_H 3.03) with H-9*β* (δ_H 4.16), of H-9*α* (δ_H 3.77) with H-7 (δ_H 4.60) and H-7′ (δ_H 4.62). By comparing of the CD of **2** with those of terminaloside I [11], they displayed the same Cotton effects, suggesting the absolute configuration of **2** corresponded to (7*R*, 8*S*, 7′*R*, 8′*S*). Thereby, the structure of **2** was determined and named buxruguloside B.

Compound **3**, isolated as yellow gum, had the molecular formula $C_{31}H_{43}NO_{11}$ based on the HR-ESIMS spectrum (*m*/*z* 606.2905, [M + H]$^+$, Calcd. 606.2909). Comparation the

Table 2 NMR Data of 2–4 in $CDCl_3$ (δ in ppm, *J* in Hz)

Position	**2** [a]		**3** [b]		**4** [a]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	130.6		131.0		130.5	
2	108.8	6.73, d (1.6)	109.0	6.78, br s [c]	111.4	6.59, br s
3	148.1		148.2		148.0	
4	147.5		147.6		146.1	
5	122.0		121.9		122.2	
6	118.2	6.51, d (1.6)	118.5	6.57, br s	120.5	6.39, br s
7	86.1	4.60, d (5.5)	88.1	4.33, d (5.5)	33.2	2.42, m; 2.85, dd (13.7, 4.4)
8	54.0	3.03, m	54.4	2.87, m	42.8	2.65, m
9	71.8	3.77 [c]; 4.16 [c]	71.2	3.77 [c]; 4.06, d (9.8)	73.2	3.72 [c]; 3.98, m
1′	136.1		134.0		140.8	
2′	110.0	6.81, d (1.8)	109.9	6.90, br s	103.2	6.53 [c], br s
3′	149.2		149.4		152.7	
4′	145.7		145.3		134.1	
5′	116.5	6.96, d (8.3)	117.3	7.01, d (8.0)	152.7	
6′	118.8	6.77, d (8.2)	118.3	6.77, d (7.6) [c]	103.2	6.53 [c], br s
7′	85.7	4.62, d (5.4)	82.0	4.78, d (5.4)	83.0	4.76, d (5.2)
8′	54.4	2.99, m	50.2	3.29, m	52.8	2.36, m
9′	71.4	3.77 [c]; 4.16 [c]	69.8	3.21, m; 3.79 [c]	60.6	3.86, m
3-OCH_3	56.1	3.82, s	56.2	3.85, s	56.1	3.81, s
3'-OCH_3	56.4	3.70, s	56.1	3.77, s	56.5	3.75 [c], s
5'-OCH_3					56.5	3.75 [c], s
1″	101.4	4.81, d (7.3)	102.1	4.85 d, (5.4)	105.6	4.62, d (5.2)
2″	73.1	3.78, m	73.4	3.72, m	74.3	3.98, m
3″	76.0	3.57, m	76.2	3.69, m	76.4	3.63, m
4″	69.2	3.63, m	69.6	3.71, m	69.9	3.63, m
5″	76.0	3.61, m	76.3	3.69, m	76.3	3.30, m
6″	61.5	3.76 [c]	61.6	3.80 [c]	61.7	3.78 [c]
5-$CH_2N(CH_2CH_3)_2$						
	57.0	3.71 [c], s	56.9	3.74, s	57.0	3.70, br s
	46.4	2.57 [c], q (7.2)	46.5	2.60 [c], q (7.2)	46.5	2.58 [c], q (7.0)
	46.4	2.57 [c], q (7.2)	46.5	2.60 [c], q (7.2)	46.5	2.58 [c], q (7.0)
	11.3	1.06 [c], t (7.2)	11.4	1.09 [c], t (7.1)	11.4	1.09 [c], t (7.0)
	11.3	1.06 [c], t (7.2)	11.4	1.09 [c], t (7.1)	11.4	1.09 [c], t (7.0)

[a] Recorded at 600 MHz for ^{1}H NMR and 151 MHz for ^{13}C NMR; [b] Recorded at 500 MHz for ^{1}H NMR and 126 MHz for ^{13}C NMR; [c] Signals overlapped

NMR data (Table 2) of **3** with **2** revealed that they had same substitutions in the aromatic moieties, which was confirmed by the HMBC spectrum (Fig. 3). In compound **3**, one of the methylene protons had a medium value (Δδ H9 = 0.29) and the other had a large value (Δδ H9′ = 0.58), indicating 7-H/8-H *cis* and 7′-H/8′-H *trans* [10]. This deduction was supported by the ROESY correlations (Fig. 3). The CD data of **3** were similar with those of 2-epiterminaloside D [11], indicating a (7*R*, 8*R*, 7′*S*, 8′*R*) absolute configuration. Thereby, the structure of **3** was depicted as shown and named buxruguloside C.

Compound **4** was isolated as yellow gum. The HR-ESI-MS exhibited a quasi-molecular ion peak at *m/z* 638.3167 ([M + H]$^+$, Calcd. 638.3171), indicating the molecular formula $C_{32}H_{47}NO_{12}$. The molecular mass of **4** was 32 mass units larger than **3**. The 1D NMR data (Table 2) of **4** closely resembled those of **3**, with the major differences being attributable to the chemical shifts at C-7 and C-9′. The benzylic CH_2 signals at δ_H 2.85 (1H, dd, *J* = 13.7, 4.4 Hz, H-7*α*) and 2.42 (1H, m, H-7*β*) and CH_2OH signals at δ_H 3.86 (2H, m, H-9′) indicated that compound **4** possessed a diarylepoxylignan skeleton [13]. In addition, an extra methoxyl at C-5′ appeared in **4**. This difference was verified by the HMBC correlation from CH_3O- (δ_H 3.75) to C-5′ (δ_C 152.7). The ROESY correlations of H-8 (δ_H 2.65) with H-8′ (δ_H 2.36) and H-9*β* (δ_H 3.98), of H-9*α* (δ_H 3.72) with H-7′ (δ_H 4.76) were observed,

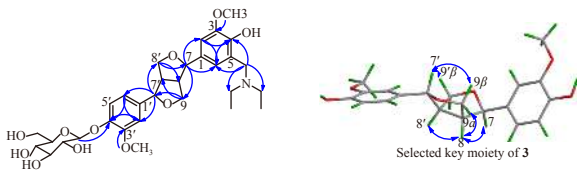

Fig. 3 Key HMBC (H → C) and ROESY (↔) correlations of 3

suggesting the opposite orientation of H-7′ and H-8, H-8′. The positive Cotton effect at 217 nm, negative Cotton effect at 237 nm and optical rotation $[\alpha]_D^{25}$ −0.2 of **4** were the same as those of alashinol B [14], suggesting that **4** had a (7′*S*, 8′*R*, 8*R*) absolute configuration. Therefore, the structure of **4** was delineated as shown and named buxruguloside D.

The absolute configuration of sugar moiety was determined by acid hydrolysis and HPLC analysis. Due to limited amounts of compounds **1** and **3**, compounds **2** and **4** were selected for acid hydrolysis and subsequent derivatization. The experimental results displayed that the retention time of the derivatives of compound **2** and **4** were highly consistent with those of the D-glucose standard. Therefore, sugars in compounds **2** and **4** were D-glucose. The corresponding HPLC analysis figure was shown in the supporting information (Fig. S50).

Compound **5**, obtained as yellow gum, afforded the molecular formula $C_{14}H_{21}NO_4$ on the basis of the HR-ESIMS ion peak at *m/z* 268.1542 ([M + H]$^+$, Calcd. 268.1543). The ^{1}H and ^{13}C spectra of **5** displayed the presence of one 1, 2, 3, 5-tetrasubstituted benzene ring [δ_H 7.37 (1H, brs, H-4) and 7.46 (1H, d, *J* = 1.6 Hz, H-6)], one methoxyl [δ_H 3.91 (3H, s)], one hydroxy group, one methyl formate group and an (*N*, *N*-diethyl)methyl amino group. According to the HMBC spectrum, the CH_3O- (δ_H 3.91) was attached to C-1 (δ_C 147.9), and the methyl formate group was connected to the C-5 (δ_C 120.1) and the (*N*, *N*-diethyl)methyl amino group was linked to C-3 (δ_C 121.3). Thus, the structure of compound **5** was defined as shown and named buxruguloside E.

In summary, buxrugulosides A–E (**1**–**5**), possessing a rare (*N*, *N*-diethyl)methyl amino group at aromatic rings, were isolated from *B. rugulosa*. Recently, two unusual aspidosperma−aspidosperma alkaloids, also bearing an (*N*, *N*-diethyl)methyl amino group at aryl group, were isolated and characterized [15]. Our present research provides additional evidence for the presence of a new class of compounds with a (*N*, *N*-diethyl)methyl amino group.

Experimental

General experimental procedures

Optical rotation values were measured on a JASCO P-1020 polarimeter (Jasco, Tokyo, Japan). UV spectra were obtained using a Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). Infrared spectra were acquired on a Bruker Tensor-27 instrument using KBr pellets (Bruker, Karlsruhe, Germany). The CD spectra in MeOH were recorded on a JASCO J-810 spectrometer (Jasco, Tokyo, Japan). HR-ESIMS data were recorded on an Agilent 6520B Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). NMR data were recorded on a Bruker AVANCE III 500 MHz or Bruker AVIIIHD 600 MHz spectrometer with tetramethylsilane (TMS) as internal standard for chemical shifts. Silica gel (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China) and RP-C_{18} (40–63 μm, FuJi Silysia Chemical Ltd., Aichi, Japan) were used for column chromatography. An Agilent 1200 Series instrument with a DAD detector using a shim-pack VP-ODS column (250 mm × 4.6 mm, 5 μm) was used for HPLC. A Shimadzu LC-6A instrument with an SPD-20A detector using a shim-packRP-C_{18} column (20 mm × 200 mm, 10 μm) was performed for further purification. TLC was performed on precoated TLC plates (F254 Si gel 60, Qingdao Marine Chemical, inc., Qingdao, China) with compounds visualized by spraying modified bismuth potassium iodide solution.

Plant material

The aerial parts of *B. rugulosa* were collected in March 2019 from Kunming, Yunnan Province, China, and authenticated by Professor ZHANG Shun-Cheng, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, China. A voucher specimen (No. BR201903) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University (Nanjing, China).

Extraction and isolation

The dried aerial parts of *B. rugulosa* (14 kg) were shattered and extracted three times (three hours each time) by refluxing with 95% ethylalcohol. The filtrate was concentrated in vacuo to yield 3.9 kg of gummy residue, which was suspended in 10% glacial acetic acid and extracted with ethylacetate. The aqueous layer was alkalinized to pH 8 with $NH_3{\cdot}H_2O$ followed by exhaustive extraction with CH_2Cl_2. The CH_2Cl_2-soluble fraction (110 g) was loaded on a silica gel column eluting with CH_2Cl_2−MeOH (100 : 0, 50 : 1, 20 : 1, 10 : 1, 2 : 1, *V/V*) containing 1% $NH(Et)_2$ to afford five fractions, Fractions A–E. Fraction D (2.03 g) was chromatographed on a MCI column eluted with aqueous MeOH (35%−85%) and ultimately gave four subfractions (Fr. D1–Fr. D4). Fr. D2 (0.77 g) was further separated by preparative HPLC (MeOH−H_2O, 43 : 57, *V/V*, 10 mL·min^{-1}) to obtain compounds **2** (64 mg), **3** (4 mg), **4** (6.2 mg), and **5** (2 mg). Fr. D3 (1.06 g) was subjected to C_{18} gel column chromatography and eluted with MeOH−H_2O. The subfraction Fr. D3c obtained using MeOH−H_2O (50 : 50, *V/V*) as the eluent was carried out on preparative HPLC (MeOH−H_2O, 40 : 60, *V/V*, 10 mL·min^{-1}) to give compound **1** (3 mg).

Spectroscopic data

Buxruguloside A (**1**): Yellow gum; $[\alpha]_D^{25}$ +6.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.03) nm; CD (MeOH, $\Delta\varepsilon$) 216 (+0.6), 238 (−0.4), 276 (−0.3) nm; IR (KBr) ν_{max} 3370, 2924, 2853, 2060, 1666, 1597, 1510, 1465, 1387, 1127, 1028, 803 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 1; HR-ESIMS *m/z* [M + H]$^+$ 832.3748 (Calcd. for $C_{42}H_{58}NO_{16}$, 832.3750).

Buxruguloside B (**2**): Yellow gum; $[\alpha]_D^{25}$ −7.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (2.91) nm; CD (MeOH, $\Delta\varepsilon$) 211 (+1.8), 230 (−0.7), 291 (+0.4) nm; IR (KBr) ν_{max} 3396, 2936, 2875, 2056, 1665, 1600, 1514, 1269, 1159, 1076 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 2; HR-ESIMS *m/z* [M + H]$^+$ 606.2904 (Calcd. for $C_{31}H_{44}NO_{11}$, 606.2909).

Buxruguloside C (**3**): Yellow gum; $[\alpha]_D^{25}$ +35.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (2.92) nm; CD

(MeOH, $\Delta\varepsilon$) 208 (+2.1), 230 (+0.7), 272 (−0.2) nm; IR (KBr) ν_{max} 3334, 2920, 2852, 1596, 1512, 1465, 1385, 1260,1225, 1075, 1037 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 2; HR-ESIMS *m/z* $[M + H]^+$ 606.2905 (Calcd. for $C_{31}H_{44}NO_{11}$, 606.2909).

Buxruguloside D (**4**): Yellow gum; $[\alpha]_D^{25}$ −0.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 (2.80) nm; CD (MeOH, $\Delta\varepsilon$) 217 (+0.4), 237 (−1.02), 277 (+0.1) nm; IR (KBr) ν_{max} 3363, 2921, 2851, 2056, 1664, 1597, 1502, 1463, 1291, 1239, 1123, 1065, 802 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 2; HR-ESIMS *m/z* $[M + H]^+$ 638.3167 (Calcd. for $C_{32}H_{48}NO_{12}$, 638.3171).

Buxruguloside E (**5**): Yellow gum; $[\alpha]_D^{25}$ −1.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (2.52) nm; IR (KBr) ν_{max} 3336, 2922, 2851, 1714, 1602, 1501, 1434, 1384, 1305, 1223, 1086, 1029, 805, 767 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37 (1H, br s, H-4), 7.46 (1H, d, 1.6, H-6), 3.91 (3H, s, 1-OCH_3), 3.87 (3H, s, 5-$COOCH_3$), 3.82 (2H, s, 3-$CH_2N(CH_2CH_3)_2$), 2.65 (4H, q, 7.1, 3-$CH_2N(CH_2CH_3)_2$), 1.13 (6H, t, 7.2, 3-$CH_2N(CH_2CH_3)_2$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 147.9 (C-1), 153.1 (C-2), 121.3 (C-3), 123.0 (C-4), 120.1 (C-5), 111.7 (C-6), 56.1 (1-OCH_3), 167.3 (5-$COOCH_3$), 52.0 (5-$COOCH_3$), 56.7 (3-$CH_2N(CH_2CH_3)_2$), 46.5 (3-$CH_2N(CH_2CH_3)_2$) × 2, 11.2 (3- $CH_2N(CH_2CH_3)_2$) × 2; HR-ESIMS *m/z* $[M + H]^+$ 268.1542 (Calcd. for $C_{14}H_{22}NO_4$, 268.1543).

Acid hydrolysis and sugar identification

The absolute configuration of the sugar unit was identified according to the procedures described in a previous report [16]. Compounds **2** and **4** (each 3 mg) were dissolved in 2 $mol \cdot L^{-1}$ HCl (6 mL) and refluxed at 100 °C for 4 h. The reaction mixture was evaporated under vacuum, and the resultant residue was partitioned between CH_2Cl_2 and H_2O. The aqueous layer was dried and dissolved in pyridine (1.5 mL) containing 5 $mg \cdot mL^{-1}$ L-cysteine methyl ester hydrochloride. After the mixture was heated at 60 °C for 2 h, *O*-tolylisothiocyanate (5 μL) was added and kept at 60 °C for additional 2 h. The reaction mixture was concentrated to dryness using a vacuum evaporator. The residue was dissolved in MeOH and analyzed by reversed-phase HPLC using 25% ACN containing 0.1% formic acid for 40 min at a flow rate 0.8 $mL \cdot min^{-1}$, and UV detection wavelength was set at 254 nm. D-Glucose in the acid hydrolysates of compounds **2** (t_R 22.993 min) and **4** (t_R 22.914 min) was identified by comparison of the retention time of derivatives with those of authentic D-glucose (t_R 22.828 min) prepared in the same procedures.

Supporting information

Supporting information of this paper can be requested by sending E-mails to the corresponding authors.

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