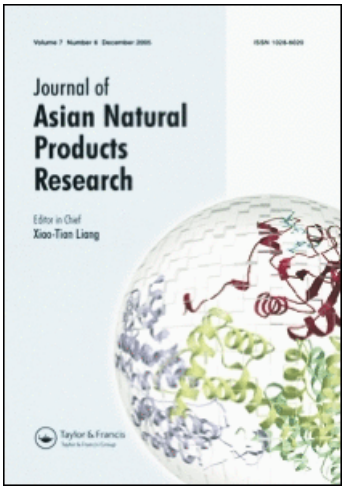

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Norsesquiterpenoid glucosides and a rhamnoside of pyrrolizidine alkaloid from *Tephroseris kirilowii*

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Four new glycosylated compounds have been isolated from the whole plant of *Tephroseris kirilowii*, including (−)-(1*R*,5*R*,6*S*,7*R*,8*S*)-8-*O*-β-D-glucopyranosyloxy-7-hydroxy-6-(2-hydroxypropan-2-yl)-9-methylenebicyclo[4.3.0]non-3-one (tephroside A, **1**), (−)-(1*R*,5*R*,6*R*,8*R*)-6-(2-*O*-β-D-glucopyranosyloxypropan-2-yl)-8-hydroxy-9-methylenebicyclo[4.3.0]non-3-one (tephroside B, **2**), thesinine-4′-*O*-α-L-rhamnoside (**3**), and *p*-coumaric acid 4-*O*-α-L-rhamnoside (**4**), together with the known roseoside. The structures of the new compounds were established by means of spectroscopic analysis.

Keywords: *Tephroseris kirilowii*; Compositae; Sesquiterpenoids; Pyrrolizidine alkaloid; Oplopane; Tephroside

1. Introduction

Tephroseris kirilowii (Turcz.) Holub. (Compositae) is a perennial herb widely distributed in China [1]. The whole plant of *T. kirilowii* is used to treat urethral infection, oedema, eczema, scabies, vaginal trichomoniasis, and leukaemia in Chinese-folk medicine [2]. In an earlier work, a pyrrolizidine alkaloid O^7-angeloyl-heliotridine showing weak activity against leukaemia L_{1210} cell has been isolated from the plant [3]. Our continuing study on the constituents of the whole plant of *T. kirilowii* led to the isolation of four new compounds, including two bisnorsesquiterpenoid glucosides of oplopane derivatives (−)-(1*R*,5*R*,6*S*,7*R*,8*S*)-8-*O*-β-D-glucopyranosyloxy-7-hydroxy-6-(2-hydroxypropan-2-yl)-9-methylenebicyclo[4.3.0]non-3-one (tephroside A, **1**), (−)-(1*R*,5*R*,6*R*,8*R*)-6-(2-*O*-β-D-glucopyranosyloxypropan-2-yl)-8-hydroxy-9-methylenebicyclo[4.3.0]non-3-one (tephroside B, **2**), a rhamnoside of pyrrolizidine alkaloid thesinine-4′-*O*-α-L-rhamnoside (**3**), and a phenylpropanoid rhamnoside *p*-coumaric acid 4-*O*-α-L-rhamnoside (**4**), together with a known bisnorsesquiterpenoid glucoside roseoside

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[4] (figure 1). As far as we know, there is only one report about bisnorsesquiterpenoids of oplopane derivative [5] and one about pyrrolizidine alkaloid glycoside [6] so far. Furthermore, compounds **1** and **2**, this type of sesquiterpenoids, were firstly isolated from higher plants. This paper reports the structural elucidation of these new compounds.

2. Results and discussion

Compound **1** was obtained as colourless amorphous gum. The molecular formula of **1** was deduced as $C_{19}H_{30}O_9$ by the $[M—H]^-$ ion peak at *m/z* 401.1822 in the HRESI-MS, which possessed 5 degrees of unsaturation. The IR spectrum of **1** showed absorption bands for hydroxyl groups (3417 cm^{-1}) and ketone functionality (1739 cm^{-1}). Its ^{13}C NMR spectrum (table 1) exhibited 19 signals including three quaternary carbons, ten methines, four methylenes, and two methyl groups. A carbonyl [δ 219.3 (s)] and an *exo*-double bond [δ 148.4 (s), 109.7 (t)] accounted for 2 degrees of unsaturation. The remaining 3 degrees of unsaturation required a tricyclic ring system in **1**.

After acidic hydrolysis of **1**, a D-glucose was obtained and detected by TLC and optical rotation, $[\alpha]_D^{17}$ + 40.3 (H_2O, *c* 0.88). The anomeric proton signal at δ 4.36 (d, J = 7.7 Hz) in the ^1H NMR spectrum indicated that the glucose was in the β-glycoside form. Besides the glucosyl moiety, the remaining fragment contained 13 carbons with two rings. The signals of C-7 at δ 76.4, C-8 at δ 86.5, and C-11 at δ 75.0 indicated the presence of three oxygen-bearing carbons in the fragment. The $^1H—^1H$ COSY spectrum revealed a connectivity of C-2—C-1—C-5—(C-4)—C-6—C-7—C-8 (figure 3). Considering this connectivity, the HMBC correlations (figure 2) of H-1/C-3, H-5/C-3, H_2-2/C-4, and H_2-4/C-2 allowed the presence of a cyclopentanone moiety, H_2-10/C-9, C-1 and C-8 revealed a six-membered ring moiety bearing an *exo*-double bond at C-9, H_3-12/C-11 and C-6, and H_3-13/C-11 and C-6 showed a (2-hydroxyl)-isopropyl moiety at C-6, and H-1′/C-8 indicated the β-D-glucosyl moiety was linked at C-8. Then, the planar structure of **1** was established as a bisnorsesquiterpenoid glucoside of oplopane derivative.

The relative configuration of **1** was elucidated by analysis of the coupling constants and ROESY correlations. In the ^1H NMR spectrum, the vicinal large coupling constants between H-4_α and H-5 ($J_{4\alpha-5}$ = 12.3 Hz), and H-6 and H-7 (J_{6-7} = 9.2 Hz) were characteristics of *trans* diaxial relationships [5], and the small one between H-7 and H-8 (J_{7-8} = 2.8 Hz) indicated that the orientation of H-8 was equatorial. The ROESY correlations of H-1/H-2_α, H-1/H-4_α, H-1/H-6, H-5/H-2_β, H-5/H-4_β, and H-5/H-7 (figure 3) implied the fusion of the five-numbered ring and the six-numbered ring was *trans* and the six-numbered ring was in chair form.

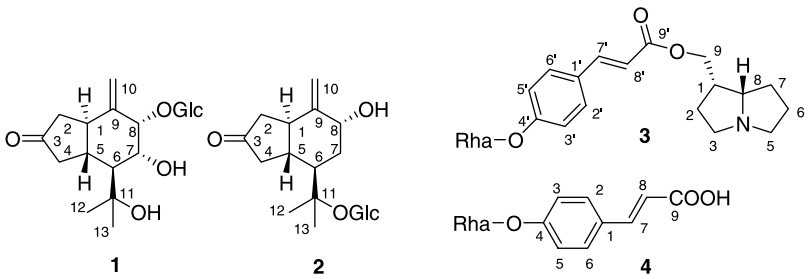

Figure 1. Structures of compounds **1**–**4**.

Table 1. ^{1}H (500 MHz) and ^{13}C (100 MHz) NMR data for **1** and **2** in CD_3OD (δ in ppm, J in Hz).

	1		***2***	
No.	δ_H	δ_C	δ_H	δ_C
1	3.10 (m)	44.2	2.83 (m)	44.1
2α	2.29 (dd, $J = 17.7, 6.5$)	41.1	2.25 (dd, $J = 17.8, 6.5$)	41.8
2β	2.14 (m)		2.14 (dd, $J = 17.8, 13.7$)	
3		219.3		221.3
4α	2.22 (dd, $J = 17.8, 12.3$)	48.0	2.44 (dd, $J = 18.6,12.4$)	48.0
4β	2.55 (dd, $J = 17.8, 6.4$)		2.73 (dd, $J = 18.6, 6.5$)	
5	1.58 (m)	44.0	1.63 (m)	47.8
6	2.14 (m)	53.3	2.30 (ddd, $J = 13.6, 13.6, 3.0$)	46.8
7α		76.4	2.11 (ddd, $J = 13.6, 3.0, 3.0$)	37.7
7β	3.85 (dd, $J = 9.2, 2.8$)		1.39 (ddd, $J = 13.6, 13.6, 3.0$)	
8	4.32 (d, $J = 2.8$)	86.5	4.38 (br. d, $J = 3.0$)	73.4
9		148.4		152.1
10	5.13 (s)	109.7	4.94 (s)	107.4
	4.81 (s)		4.68 (s)	
11		75.0		81.2
12	1.28 (s)	26.7	1.15 (s)	22.7
13	1.24 (s)	30.2	1.28 (s)	25.7
1′	4.36 (d, $J = 7.7$)	104.4	4.51 (d, $J = 7.7$)	98.5
2′	3.28 (dd, $J = 8.5, 7.7$)	75.3	3.13 (dd, $J = 9.0, 7.7$)	75.4
3′	3.37 (t, $J = 8.5$)	77.9	3.33 (m)	78.6
4′	3.35 (m)	71.3	3.24 (m)	71.8
5′	3.13 (m)	77.8	3.24 (m)	77.6
6′	3.70 (dd, $J = 11.9, 2.1$)	62.4	3.82 (d, $J = 12.0$)	63.0
	3.63 (dd, $J = 11.9, 4.9$)		3.62 (dd, $J = 12.0, 4.6$)	

The absolute configuration of **1** was proposed from the CD spectrum. Based on the octant rule for the cyclopentanone [7], the negative Cotton effect at 289 nm suggested that the configuration of **1** is as depicted in figure 3. Thus, the structure of **1** was determined as (−)-(1*R*,5*R*,6*S*,7*R*,8*S*)-8-*O*-β-D-glucopyranosyloxy-7-hydroxy-6-(2-hydroxypropan-2-yl)-9-methylenebicyclo[4.3.0]non-3-one, named tephroside A.

Compound **2** was obtained as colourless amorphous gum. The molecular formula of **2** was deduced as $C_{19}H_{30}O_8$ by the $[M + Na]^+$ ion peak at m/z 409.1842 in the HRESI-MS, which possessed 5 degrees of unsaturation. The IR spectrum of **2** showed absorption bands for hydroxyl groups (3418 cm^{-1}) and ketone functionality (1728 cm^{-1}).

After a detailed examination of 1D NMR spectroscopic data (table 1), 2D NMR correlations (figures 2 and 3), and MS spectra of **2** and **1**, it was observed that **2** was similar to **1**, except that C-7 [δ 37.7 (t)] of **2** was not substituted by the hydroxyl, and the glucosyl moiety of **2** was linked at C-11 [δ 81.2 (s)] instead of C-8 in compound **1**, which was confirmed by the correlation of H-1′/C-11 in the HMBC spectrum of **2** (figure 2).

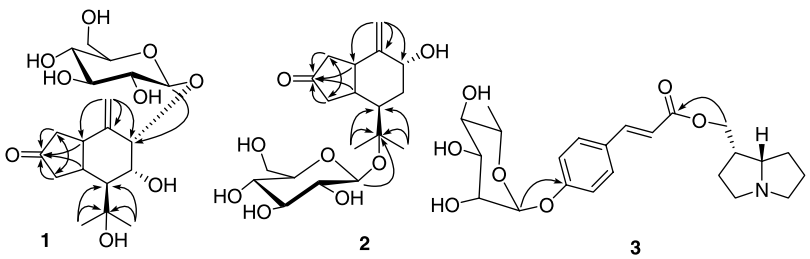

Figure 2. Significant HMBC correlations for **1**–**3**.

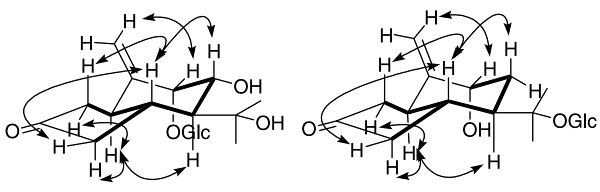

Figure 3. Key $^{1}H—^{1}H$ COSY (bold) and ROESY (arrows) correlations for **1** and **2**.

As shown in figure 3, the relative configuration of **2** was established by analysis of the coupling constants and ROESY correlations, and the absolute configuration of **2** was elucidated by the CD spectrum, respectively. Accordingly, **2** was determined as (−)-(1*R*,5*R*,6*R*,8*R*)-6-(2-*O*-β-D-glucopyranosyloxypropan-2-yl)-8-hydroxy-9-methylenebicyclo[4.3.0]non-3-one, named tephroside B.

Compound **3** was obtained as colourless amorphous solid. The molecular formula of **3** was determined as $C_{23}H_{31}NO_7$ by the HRESI-MS, which exhibited a $[M + H]^+$ ion peak at *m/z* 434.2190. The IR spectrum of **3** showed absorption bands for hydroxyl group (3431 cm^{-1}), conjugated carbonyl (1713 and 1632 cm^{-1}) and phenyl ring (1603 and 1510 cm^{-1}). Analysis of ^{1}H NMR and ^{13}C NMR data and the HSQC spectrum of **3** revealed the presence of three quaternary carbons, 13 methines, six methylenes, and one methyl group. Among them, signals at δ_C 99.7 (d), 73.7 (d), 72.2 (d), 71.9 (d), 70.9 (d), 18.0 (q) and an anomeric proton resonance at δ 5.49 (d, $J = 1.2$ Hz) were the characteristics of α-rhamnosides [8]. By comparison of the ^{1}H NMR and ^{13}C NMR data of **3** with those of thesinine-4′-*O*-β-D-glucoside published in the literature [6], a thesinine moiety, comprising a *p*-coumaric group [δ_H 7.59 (d, $J = 8.6$ Hz), 7.11 (d, $J = 8.6$ Hz), 7.67 (d, $J = 15.9$ Hz), 6.44 (d, $J = 15.9$ Hz); δ_C 168.4 (s)] and a 1-hydroxymethyl pyrrolizidine residue [δ 69.5 (d), 41.4 (d), 64.4 (t), 57.0 (t), 54.9 (t), 27.3 (t), 27.0 (t), and 26.9 (t)], could be affirmed. The HMBC showed correlation between the anomeric proton and C-4′. Thus, the structure of compound **3** was elucidated as thesinine-4′-*O*-α-L-rhamnoside.

Compound **4** was obtained as colourless amorphous solid. The molecular formula of **4** was determined as $C_{15}H_{18}O_7$ by the HRESI-MS, which exhibited a $[M—H]^-$ ion peak at *m/z* 309.0982. According to the NMR data of **4**, there was an α-rhamnose moiety [δ_H 5.46 (br. s); δ_C 99.8 (d), 73.9 (d), 72.3 (d), 72.0 (d), 70.8 (d), 18.0 (q)] in **4**. A *p*-coumaric group was deduced by the signal at δ_C 175.4 (s, C-9) in the ^{13}C NMR spectrum, a set of AA′XX′ doublets at δ 7.48 (d, $J = 8.6$ Hz) and 7.06 (d, $J = 8.6$ Hz), and two *trans* olefinic protons at δ 7.40 (d, $J = 15.9$ Hz) and 6.39 (d, $J = 15.9$ Hz) in the ^{1}H NMR spectrum [6]. The HMBC showed correlation between the anomeric proton and C-4. Thus, the structure of compound **4** was elucidated as *p*-coumaric acid 4-*O*-α-L-rhamnoside.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a Jasco DIP-370 digital polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 infrared spectrophotometer. UV spectra were recorded on a Shimadzu double-beam 210A spectrometer. CD spectra were recorded on a Jasco J-810

spectropolarimeter. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers. MS were measured on a VG Auto Spec-3000 mass spectrometer. Column chromatography (CC) was performed over silica gel (200–300 and 300–400 mesh; Qingdao Haiyang Chem. Ind. Co. Ltd.) and Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden). TLC was performed on precoated plates with silica gel F_{254} (Qingdao Haiyang Chem. Ind. Co. Ltd.).

3.2 Plant material

Tephroseris kirilowii was collected from Guanshan Pasture, Longxian County, Shanxi Province of China, in August 1999. The plant was identified by associate Professor Jin-Xiang Yang, Northwest A&F University, Shanxi, China, and a voucher specimen (No. GS 9901) is deposited at the laboratory in the College of Animal Science and Technology, Northwest A&F University, Shanxi, China.

3.3 Extraction and isolation

The air-dried whole plant of *T. kirilowii* (10 kg) was milled and extracted with MeOH at room temperature to give a crude extract. The crude extract (770 g) was dissolved in 1% HCl to form a suspension and adjusted to pH 3. The acidic suspension was immediately partitioned with CH_2Cl_2. Then, the acidic aqueous phase was adjusted with 25% ammonia to pH 10 and partitioned with CH_2Cl_2 to give the crude alkaloids (6.7 g). The crude alkaloids were separated by silica gel CC eluted with $CHCl_3$/MeOH/Et_2NH (80:10:1) and purified by Sephadex LH-20 CC ($CHCl_3$/MeOH, 1:1) to yield **3** (5 mg) and **4** (3 mg). The basic aqueous phase after being partitioned by CH_2Cl_2 was sequentially extracted with *n*-BuOH to give a residue (30 g). The residue was chromatographed over silica gel using $CHCl_3$/MeOH (5:1) to afford a major fraction. The fraction was separated by silica gel CC eluted with $CHCl_3$/MeOH/H_2O (92:7:1) and ($CHCl_3$/Me_2CO, 3:1 and 1:1) to yield **1** (100 mg), **2** (17 mg), and roseoside (10 mg).

3.3.1 Tephroside A (1). Colourless amorphous gum (MeOH); $[\alpha]_D^{23}$ −100.3 (MeOH, *c* 2.37); UV λ_{max} (MeOH, nm, log ε): 353 (1.86), 328 (1.95), 289 (2.19); IR ν_{max} (KBr, cm^{-1}): 3417, 1739, 1077, 1053, 910; CD (MeOH, nm, $\delta\varepsilon_{max}$): 289 (−2.08); FAB-MS *m/z* 401 $[M-H]^-$; HRESI-MS *m/z* 401.1822 $[M-H]^-$ (calcd for $C_{19}H_{29}O_9$, 401.1811); ^1H NMR and ^{13}C NMR spectral data: see table 1.

3.3.2 Tephroside B (2). Colourless amorphous gum (MeOH); $[\alpha]_D^{23}$ −78.2 (MeOH, *c* 0.72). UV λ_{max} (MeOH, nm, log ε): 289 (2.35); IR ν_{max} (KBr, cm^{-1}): 3418, 1728, 1078, 1031, 904; CD (MeOH, nm, $\delta\varepsilon_{max}$): 289 (−5.97); HRESI-MS *m/z* 409.1842 $[M + Na]^+$ (calcd for $C_{19}H_{30}O_8Na$, 409.1838); ^1H NMR and ^{13}C NMR spectral data: see table 1.

3.3.3 Thesinine-4′-*O*-α-L-rhamnoside (3). Colourless amorphous solid; $[\alpha]_D^{23}$ −72.7 (MeOH, *c* 0.55); UV λ_{max} (MeOH, nm, log ε): 298 (4.22), 220 (4.22); IR ν_{max} (KBr, cm^{-1}): 3431, 1713, 1632, 1603, 1510, 1241, 1010, 979; ESI-MS *m/z* 434 $[M + H]^+$; HRESI-MS

Table 2. ^{1}H (500 MHz) and ^{13}C (100 MHz) NMR data for **3** in CD_3OD (δ in ppm, J in Hz).

No.	δ_H	δ_C	*No.*	δ_H	δ_C
1	2.75 (m)	41.4	4′		159.8
2	2.08 (m)	27.3†	7′	7.67 (d, $J = 15.9$)	146.3
	1.91 (m)				
3	3.36 (m)	54.9	8′	6.44 (d, $J = 15.9$)	116.5
	3.13 (m)				
5	3.60 (m)	57.0	9′		168.4
	2.88 (ddd, $J = 15.4, 5.9, 5.9$)				
6	1.81 (m)	26.9†	1″	5.49 (d, $J = 1.2$)	99.7
	2.08 (m)				
7	1.81 (m)	27.0†	2″	4.0 (br. d, $J = 1.2$)	71.9
	2.02 (m)				
8	4.05 (m)	69.5	3″	3.83 (dd, $J = 9.5, 3.4$)	72.2
9	4.35 (dd, $J = 11.2, 6.6$)	64.4	4″	3.48 (t, $J = 9.5$)	73.7
	4.26 (dd, $J = 11.2, 8.2$)				
1′		129.6	5″	3.59 (m)	70.9
2′/6′	7.59 (d, $J = 8.6$)	131.0	6″	1.22 (d, $J = 6.1$)	18.0
3′/5′	7.11 (d, $J = 8.6$)	117.8			

† Entries with the same superscript are interchangeable.

m/z 434.2190 $[M + H]^+$ (calcd for $C_{23}H_{32}NO_7$, 434.2178); ^{1}H NMR and ^{13}C NMR spectral data: see table 2.

3.3.4 *p*-Coumaric acid 4-*O*-α-L-rhamnoside (4). Colourless amorphous solid; $[\alpha]_D^{23}$ − 104.6 (MeOH, *c* 0.36); UV λ_{max} (MeOH, nm, log ε): 272 (4.14); IR ν_{max} (KBr, cm^{-1}): 3424, 1634, 1605, 1509, 1237, 1018, 981; ESI-MS *m/z* 309 $[M-H]^-$; HRESI-MS *m/z* 309.0982 $[M-H]^-$ (calcd for $C_{15}H_{17}O_7$, 309.0974); ^{1}H NMR (500 MHz, CD_3OD): 7.48 (d, $J = 8.6$ Hz, H-2, H-6), 7.06 (d, $J = 8.6$ Hz, H-3, H-5), 7.40 (d, $J = 15.9$ Hz, H-7), 6.39 (d, $J = 15.9$ Hz, H-8), 5.46 (br. s, H-1′), 4.00 (br. d, $J = 1.1$ Hz, H-2′), 3.84 (dd, $J = 9.5$, 3.3 Hz, H-3′), 3.45 (t, $J = 9.5$ Hz, H-4′), 3.61 (dd, $J = 9.5$, 6.2 Hz, H-5′), 1.22 (d, $J = 6.2$ Hz, H-6′); ^{13}C NMR (100 MHz, CD_3OD): 131.3 (s, C-1), 130.0 (d, C-2, C-6), 117.7 (d, C-3, C-5), 158.7 (s, C-4), 141.2 (d, C-7), 123.9 (d, C-8), 175.4 (s, C-9), 99.8 (d, C-1′), 72.0 (d, C-2′), 72.3 (d, C-3′), 73.9 (d, C-4′), 70.8 (d, C-5′), 18.0 (q, C-6′).

3.4 Acid hydrolysis of 1

Compound **1** (40 mg) was dissolved in 25 ml of 6% aq. HCl and hydrolysed under reflux (2 h) at 90°C. Then, the acidic solution was evaporated *in vacuo* to dryness and separated by silica gel CC eluted with $CHCl_3$/MeOH (3:1) to yield 7 mg of D-glucose detected by TLC and optical rotation, $[\alpha]_D^{17}$ + 40.3 (H_2O, 0.88).

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