SUPPLEMENTARY MATERIAL

Chemical Constituents Isolated from *Zygophyllum melongena* Bunge Growing in Mongolia

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Abstract: We report the first investigation of the chemical constituents of *Zygophyllum melongena* Bunge, a species growing in Mongolia. The quinovic acid glycosides 3-*O*-(β -D-glucopyranosyl)quinovic acid and 3-*O*-(β -D-glucopyranosyl)quinovic acid (28 $\rightarrow$ 1)-(β -D-glucopyranosyl) ester were identified in the chloroform fraction along with the flavonoid glycoside astragalin. The *n*-butanol fraction contained (+)-D-pinitol, a cyclitol with anti-diabetic properties, as the major component. The structures of the isolated natural products were confirmed using ESI-MS and NMR spectroscopy (¹H, ¹³C, COSY, HSQC, HMBC, NOESY and ROESY). This is the first report of the isolation of (+)-D-pinitol from the genus *Zygophyllum*.

Keywords: Astragalin; *Zygophyllum melongena*; NMR spectroscopy; quinovic acid; pinitol

Experimental section

General experimental procedure

The NMR spectra were recorded on a Bruker AVANCE III 600 MHz spectrometer using methanol- d_4 as solvent. The chemical shifts δ are reported in ppm using the non-deuterated residual solvent as internal standard ($\delta_H = 3.35$, $\delta_C = 49.00$). Coupling constants J are given in Hz. Complete assignments of the 1H NMR and ^{13}C NMR signals were achieved by measuring the following 2D NMR spectra: COSY, HSQC, HMBC, NOESY, and ROESY. The mass spectra and high resolution mass spectra were recorded on a Finnigan/MAT-95 (electron impact, 70 eV). ESI-MS spectra were recorded on a Bruker-Esquire LC ion trap detector; positive and negative ions were detected. Optical rotation was measured with a Perkin-Elmer 341 polarimeter at a wavelength of 589 nm (sodium D line) using a 1.0-decimeter cell with a total volume of 1.0 mL. Thin layer chromatography was performed on silica gel 60 F₂₅₄ (Merck). Preparative TLC was performed with glass plates (20×20 cm, Merck) coated with a 0.25 mm layer of silica gel (60-F₂₅₄), mobile phase $CHCl_3/MeOH$ (3:1). Column chromatography was performed using silica gel (0.040–0.063 mm, Merck) and Sephadex LH-20. Sequential treatment with iodine vapours or treatment with sulfuric acid (5 vol.-% in ethanol) and subsequent heating with a hot air gun were used for visualization.

Plant material

The aerial parts of *Zygophyllum melongena* Bunge were collected in August 2010 from Bayankhongor aimag, Bayantsagaan sum, Mongolia. The plant was identified by Prof. Dr. Chinbat Sanchir at the herbarium of the Ulaanbaatar Institute of Botany, Mongolian Academy of Sciences, where a voucher specimen has been deposited (voucher number: chl.08.2010).

Extraction and isolation

The air-dried and powdered aerial parts of the plant (4.0 kg) were exhaustively extracted with 96% ethanol at room temperature. The extracts were combined and concentrated under reduced pressure to leave a residue, which was suspended in H_2O and then fractioned by re-extracting with *n*-hexane (101.7 g), chloroform (50.6 g), ethyl acetate (15.1 g), and *n*-butanol (113.6 g), respectively. The chloroform extract (44.0 g) was subjected to column chromatography on silica gel (0.040–0.063 mm, Merck), eluted with $CHCl_3/MeOH$ (gradient from 100:0 to 85:15) to afford 190 fractions based on the TLC profiles. The fractions 137–141 (358 mg) afforded colorless crystals. The colorless crystals were collected by filtration to yield compound **1** (46 mg). A small portion of the crystals was dissolved in methanol and analyzed by TLC (eluent: $CHCl_3/MeOH$, 4:1) to confirm the purity. The fractions 169–179 were combined and separated by using preparative TLC with $CHCl_3/MeOH$ (3:1) as eluent to obtain compound **2** (21.5 mg).

The fractions 151–156 were purified at Sephadex LH-20 by elution with $\text{CHCl}_3/\text{MeOH}$ (90:10) to afford compound **3** (13 mg). The *n*-butanol extract (113.6 g) was dissolved in methanol; upon heating a colorless precipitate was formed. The colorless precipitate (6.0 g) was removed by filtration. The filtrate was concentrated under reduced pressure to leave a residue (92.2 g), 46.0 g of which were subjected to column chromatography on silica gel and eluted with $\text{CHCl}_3/\text{MeOH}$ (gradient from 100:0 to 85:15) to afford 171 fractions based on the TLC profiles. The fractions 125–127 and 133–135 afforded a colorless precipitate. The colorless precipitate was collected by filtration to provide compound **4** (3.49 g).

Table S1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data of **1** and **2** (solvent: methanol- d_4).^a

Position	1		2	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
Aglycone				
1	39.94	1.72, m; 1.07, m	39.71	1.69, m; 1.13, m
2	27.08	1.98, m; 1.74, m	27.15	1.96, m; 1.75, m
3	90.66	3.19, m	91.05	3.17, dt (11.0, 4.5)
4	40.10	—	40.14	—
5	56.92	0.79, dd (11.7, 1.1)	56.55	0.87, m
6	19.28	1.57, m; 1.39, m	19.39	1.53, m; 1.36, m
7	38.04	1.69, m; 1.26, m	38.10 ^c	1.62, m; 1.42, m
8	40.66	—	40.55	—
9	48.03	2.28, dd (11.9, 5.3)	47.70	2.46, dd (11.3, 5.0)
10	37.84	—	37.90	—
11	23.84	2.03, m; 1.94, m	24.12	1.99, m; 1.91, m
12	130.44	5.65, dd (5.3, 2.5)	129.52 ^c	5.57, m
13	133.88	—	134.81 ^b	—
14	57.28	—	58.76 ^b	—
15	25.74	2.09, m; 1.77, m	27.00	2.21, m; 1.75, m
16	26.46	2.10, m; 1.71, m	26.33	2.11, m; 1.70, m
17	49.42 ^b	—	50.09 ^b	—
18	55.53	2.29, d (10.9)	55.68	2.27, d (11.5)
19	38.31	1.04, m	38.04	1.21, m
20	40.39	1.01, m	40.45	0.98, m
21	31.22	1.49, m; 1.31, m	31.34 ^c	1.48, m; 1.35, m
22	37.63	1.69, m	37.34	1.75, m
		1.64, dt (3.9, 13.4)		1.62, m
23-H ₃	28.49	1.06, s	28.52	1.05, s
24-H ₃	17.05	0.87, s	17.11	0.86, s
25-H ₃	16.89	1.02, s	17.02	1.00, s
26-H ₃	19.07	0.94, s	19.19	0.89, s
27	179.00	—	179.17 ^b	—
28	181.59	—	178.31 ^b	—
29-H ₃	18.14	0.94, d (5.6)	18.60	0.98, d (6.2)
30-H ₃	21.51	0.96, d (5.6)	21.47	0.96, br. s
glucose at C-3				
1'	106.69	4.34, d (7.8)	106.74	4.33, d (7.8)
2'	75.67	3.22, dd (8.9, 7.9)	75.70	3.22, m
3'	78.27	3.37, t (8.9)	78.29	3.37, m
4'	71.67	3.32, m	71.64	3.33, m
5'	77.69	3.28, m	77.67	3.27, m
6'	62.81	3.88, dd (12.4, 2.4)	62.78	3.85, m
		3.70, dd (12.0, 5.4)		3.71, m
glucose at C-28				
1''			95.61	5.42, d (8.2)
2''			73.94	3.36, m
3''			78.36	3.44, m
4''			71.26	3.39, m
5''			78.62	3.38, m
6''			62.61	3.84, m; 3.71, m

^a Numbering of the compound framework, see: Figure 1.^b Assignment based on the HMBC spectrum.^c Assignment based on the HSQC spectrum.

Table S2. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data of astragalin (**3**) and (+)-D-pinitol (**4**) (solvent: methanol- d_4).^a

Position ^a	3		4	
	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)
1			73.77 ^d	3.92, m
2	158.98 ^b		72.05	3.78, dd (9.7, 2.3)
3	—		84.95	3.29, t (9.5)
4	—		74.34	3.63, t (9.5)
5	—		72.56	3.73, dd (9.7, 2.3)
6	100.0 ^c	6.25, d (2.0)	73.49 ^d	3.92, m
OCH ₃			60.82	3.65, s
7	—			
8	94.79 ^c	6.45, d (2.0)		
9	158.61 ^b			
10	105.66 ^b			
1'	122.79 ^b			
2'/6'	132.31 ^c	8.10, d (8.9)		
3'/5'	116.10 ^c	6.93, d (8.9)		
4'	161.38 ^b			
3-glucosyl				
1''	104.10 ^c	5.30, d (7.5)		
2''	75.76 ^c	3.47, m		
3''	78.10 ^c	3.46, m		
4''	71.40 ^c	3.37, m		
5''	78.44 ^c	3.23, m		
6''	62.64 ^c	3.73, dd (11.9, 2.2) 3.57, dd (11.9, 5.6)		

^a Numbering of the compound frameworks, see: Figure 1.

^b Assignment based on the HMBC spectrum.

^c Assignment based on the HSQC spectrum.

^d Assignment of C-1 and C-6 is ambiguous and may be interchanged.

— not visible due to the low concentration of compound **3**.

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Figure S1. The ¹H NMR Spectrum of Compound 1 in Methanol-*d*₄ (600 MHz)

Figure S2. The ¹³C NMR Spectrum of Compound 1 in Methanol-*d*₄ (150 MHz)

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Figure S4. The COSY Spectrum of Compound 1 in Methanol- d_4

Figure S5. The COSY Spectrum of Compound 1 in Methanol- d_4

Figure S6. The HSQC Spectrum of Compound 1 in Methanol- d_4

Figure S7. The HSQC Spectrum of Compound 1 in Methanol- d_4

Figure S8. The HSQC Spectrum of Compound 1 in Methanol- d_4

Figure S9. The HMBC Spectrum of Compound 1 in Methanol- d_4

Figure S10. The HMBC Spectrum of Compound 1 in Methanol- d_4

Figure S11. The HMBC Spectrum of Compound 1 in Methanol- d_4

Figure S12. The HMBC Spectrum of Compound 1 in Methanol- d_4

Figure S13. The ROESY Spectrum of Compound 1 in Methanol- d_4

Figure S14. The ROESY Spectrum of Compound 1 in Methanol- d_4

Figure S15. The ROESY Spectrum of Compound 1 in Methanol- d_4

Figure S16. The ^1H NMR Spectrum of Compound 2 in Methanol- d_4 (600 MHz)

Figure S17. The ¹³C NMR Spectrum of Compound 2 in Methanol-*d*₄ (150 MHz)

Figure S18. The COSY Spectrum of Compound 2 in Methanol- d_4

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Figure S20. The COSY Spectrum of Compound 2 in Methanol- d_4

Figure S22. The HSQC Spectrum of Compound 2 in Methanol- d_4

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Figure S24. The HMBC Spectrum of Compound 2 in Methanol- d_4

Figure S25. The HMBC Spectrum of Compound 2 in Methanol- d_4

Figure S26. The HMBC Spectrum of Compound 2 in Methanol- d_4

Figure S27. The NOESY Spectrum of Compound 2 in Methanol- d_4

Figure S28. The ROESY Spectrum of Compound 2 in Methanol- d_4

Figure S29. The ROESY Spectrum of Compound 2 in Methanol- d_4

Figure S30. The ROESY Spectrum of Compound 2 in Methanol- d_4

Figure S31. The DOSY Spectrum of Compound 2 in Methanol- d_4

Figure S32. The ^1H NMR Spectrum of Compound 3 in Methanol- d_4 (600 MHz)

Figure S33. The ^{13}C NMR Spectrum of Compound 3 in Methanol- d_4 (150 MHz)

Figure S34. The COSY Spectrum of Compound 3 in Methanol- d_4

Figure S35. The COSY Spectrum of Compound 3 in Methanol- d_4

Figure S36. The HSQC Spectrum of Compound 3 in Methanol- d_4

Figure S37. The HSQC Spectrum of Compound 3 in Methanol- d_4

Figure S38. The HMBC Spectrum of Compound 3 in Methanol- d_4

Figure S39. The HMBC Spectrum of Compound 3 in Methanol- d_4

Figure S40. The HMBC Spectrum of Compound 3 in Methanol- d_4

Figure S41. The NOESY Spectrum of Compound 3 in Methanol- d_4

Figure S42. The NOESY Spectrum of Compound 3 in Methanol- d_4

Figure S43. The ^1H NMR Spectrum of Compound 4 in Methanol- d_4 (600 MHz)

Figure S44. The ^{13}C NMR Spectrum of Compound 4 in Methanol- d_4 (150 MHz)

Figure S45. The COSY Spectrum of Compound 4 in Methanol- d_4

Figure S46. The HSQC Spectrum of Compound 4 in Methanol- d_4

Figure S47. The HMBC Spectrum of Compound 4 in Methanol- d_4

Figure S48. The NOESY Spectrum of Compound 4 in Methanol- d_4

Figure S49. The ROESY Spectrum of Compound 4 in Methanol- d_4