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Quinic acid esters and sesquiterpenes from *Sonchus arvensis*

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ABSTRACT

A phytochemical study on the whole plant of *Sonchus arvensis* and its antioxidant activity has been carried out. Three quinic acid derivatives (**1**–**3**), the rarely naturally occurring (*p*-hydroxyphenylacetyl) quinic acids, and two eudesmanolides (**4** and **5**) were newly found. Four known eudesmanolides (**6**–**9**) were isolated from the plant for the first time. Their structures were characterized by HRESIMS, IR, UV, 1D NMR, and 2D NMR. 1,1-Diphenyl-2-picrylhydrazyl (DPPH·)-scavenging activity was evaluated for each of the above 9 compounds (**1**–**9**) in comparison to standard antioxidants (caffeic acid and ascorbic acid). However, none proved to have a positive activity. The absence of antioxidant activity could be caused by the absence of *ortho* or *para*-diphenolic groups in all detected compounds, that are responsible of the activity against free radicals by an electron transfer reaction.

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1. Introduction

The genus *Sonchus* is composed of 8 species in China. They have long been used as folk medicines for the treatment of fever, stasis and inflammation. They also have effects on detoxification and mobilization of blood circulation (Jiangsu College of New Medicine, 1977). The plant, *Sonchus arvensis*, is valued as a delicious and nutritional potherb and has been used for the treatment of caked breasts, asthma, coughs, and other chest complaints and for calming the nerves. It also has insecticidal properties and anti-inflammatory activity (Chinese Medicines Compile Group, 1975). Flavonol (Bondarenko, Glyzin, Shelyuto, & Smirnova, 1976), flavonol glycoside (Bondarenko, Glyzin, & Shelyuto, 1978; Qu, Li, & Liu, 1996), and monoacyl galactosylglycerol (Baruah et al., 1983) were previously isolated from this plant. In the present work, three (*p*-hydroxyphenylacetyl)quinic acids (**1**–**3**) and two eudesmanolides (**4** and **5**) were newly isolated from this plant, collected in northwestern China. Four other known eudesmanolides (**6**–**9**) were also obtained from it. We now report the isolation, structural assignment and antioxidant activity assay of all the nine components.

2. Materials and methods

2.1. General methods

Optical rotations were measured on a *Perkin–Elmer Model* 341 polarimeter. IR spectra were recorded on a Nicolet Avatar 360 FT-IR instrument using KBr discs over the range of 400–4000 cm^{-1}. 1D and 2D NMR detections were conducted on a Varian Mercury-300/400bb NMR spectrometer with TMS as standards or residual solvent peak used for referencing. HRESIMS determinations were run on a Bruker APEX II FT-MS spectrometer. UV detection was measured on a Shimadzu UV-260 spectrophotometer. Analytical and preparative TLC were performed on silica gel plates (GF_{254} 10–40 μm, Qingdao Marine Chemical Factory). Analytical TLC was provided to follow the separation and check the purity of isolated compounds. Spots on the plates were observed under UV light and visualized by spraying them with 5% H_2SO_4 in C_2H_5OH (v/v), followed by heating. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Factory). Sephadex LH-20 (Amersham Pharmacia Biotech), RP-18 silica gel (150–200 mesh, Merck), and MCI gel CHP20P (75–150 μm, Mitsubishi Chemical) were used for CC. 1,1-Diphenyl-2-picrylhydrazyl (DPPH·, Fluka 43180), caffeic acid (C0625), and ascorbic acid (A5960) were purchased from Sigma (St. Louis, MO).

Abbreviations: $[\alpha]_D$, specific optical rotation at sodium D line; *c*, concentration; calcd, calculated; CC, column chromatography; COSY, correlation spectroscopy; 1D, 2D, one- or two-dimensional; DEPT, distortionless enhancement by polarization transfer; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ε, molar absorptivity; HMBC, heteronuclear multiple-bond correlation; HRESIMS, high resolution electron spray ionization mass spectrometry; *J*, coupling constant; MCI gel, MCI gel CHP20P high porous polymer (Mitsubishi Chemical Industries Co., Ltd., Japan); NOE, nuclear overhauser effect; ppm, parts per million; RP-18, reversed phase C18; TLC, thin-layer chromatography.

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2.2. Plant material

The whole plant of *S. arvensis* was collected from Zhuoni county, Gansu province, PR China, in July 2005 and identified by Dr. Hu-Yuan Feng from the College of Life Science, Lanzhou University. A voucher specimen (No. 200506SA) was deposited in the Institute of Organic Chemistry, Lanzhou University.

2.3. Extraction and isolation

Air-dried whole plant (2.2 kg) of *S. arvensis* were extracted with 95% ethanol (8 l × 7 d × 3) at room temperature. Extract (200.0 g) were obtained after removal of solvent under vacuum. Residue (200.0 g) were suspended in H_2O (0.4 l) and successively partitioned with petroleum ether (30–60 °C, 0.3 l), EtOAc (0.3 l), and *n*-BuOH (0.3 l), respectively. The EtOAc portion was concentrated under vacuum to give 8.5 g of residue, which was chromatographed on a silica gel column (200–300 mesh) using $CHCl_3$:MeOH (100:1–1:1) gradient to provide 6 fractions (Fr. 1–6). Fr. 3 was subjected to a Si-gel CC eluted with EtOAc:MeOH (100:0–1:1), to afford 6 fractions (Fr. 3–1–Fr. 3–6). **5** (6 mg) was isolated from Fr. 3–2 by a re-separation on a Si-gel CC ($CHCl_3$:MeOH, 100:1–1:1). Fr. 4 was subjected to CC eluted with EtOAc:MeOH (100:1–1:1) to give 7 fractions (Fr. 4–1–Fr. 4–7). Fr. 4–1 gave **8** (70 mg) after purification by CC on a Sephadex LH-20 column ($CHCl_3$:MeOH, 2:1). Fr. 4–3 was further purified and subjected to preparative TLC ($CHCl_3$:MeOH, 4:1) to yield **9** (15 mg). Fr. 5 was subjected to CC eluted with EtOAc:MeOH:H_2O (100:1:0.1–10:10:1) to give 6 fractions (Fr. 5–1–Fr. 5–6). Fr. 5–1 was purified by chromatography on an RP-18 column (H_2O:MeOH, 3:1–1:1) to yield **1** (10 mg) and **2** (8 mg). Fr. 5–3 gave **4** (13 mg) and **7** (35 mg) after re-separation by Si-gel CC (EtOAc:MeOH, 100:1–1:1). Fr. 5–5 was subjected to preparative TLC ($CHCl_3$:EtOAc:MeOH:H_2O, 5:5:4:1) to give **3** (3 mg). The *n*-BuOH phase was chromatographed over CC using $CHCl_3$:MeOH (100:1–1:1) to give 7 fractions (Fr. A–Fr. G). Fr. D was subjected to CC eluted with EtOAc:MeOH:H_2O (100:1:0.1–10:10:1) to give 7 fractions (Fr. D–1–Fr. D–7). Fr. D–4 was purified on MCI gel (H_2O:MeOH, 1:0–5:1) to give **6** (8 mg). The entire process is shown in Fig. 1.

1,3,4,5-tetra-(*p*-Hydroxyphenylacetyl)quinic acid (**1**): yellow gum; $[\alpha]_D^{20}$ −53 (*c* 0.02, MeOH); HRESIMS *m/z* 727.2022 (calcd for $C_{39}H_{35}O_{14}^-$, $[M-H]^-$ 727.2021); UV (MeOH) λ_{max} ($\log\varepsilon$) 225.8 (4.48), 277.0 (3.90) nm; IR (KBr) ν_{max} 3423, 1722, 1615, 1516, 1446 cm^{-1}; ^1H and ^{13}C NMR see Table 1.

1,3,4-tri-(*p*-Hydroxyphenylacetyl)quinic acid (**2**): colourless gum; $[\alpha]_D^{20}$ −52 (*c* 0.02, MeOH); HRESIMS *m/z* 617.1622 (calcd for $C_{31}H_{30}O_{12}Na^+$, $[M+Na]^+$ 617.1629); UV (MeOH) λ_{max} ($\log\varepsilon$) 225.0 (4.37), 277.4 (3.78) nm; IR (KBr) ν_{max} 3420, 1725, 1614, 1516, 1443 cm^{-1}; ^1H and ^{13}C NMR see Table 1.

3,4,5-tri-(*p*-Hydroxyphenylacetyl)quinic acid methyl ester (**3**): yellow gum; $[\alpha]_D^{20}$ −32 (*c* 0.02, MeOH); HRESIMS *m/z* 631.1781 (calcd for $C_{32}H_{32}O_{12}Na^+$, $[M+Na]^+$ 631.1786); UV (MeOH) λ_{max} ($\log\varepsilon$) 225.4 (4.32), 277.2 (3.74) nm; IR (KBr) ν_{max} 3426, 1735, 1615, 1516, 1444 cm^{-1}; ^1H and ^{13}C NMR see Table 1.

1β-Hydroxy-15-*O*-(*p*-hydroxyphenylacetyl)-5α,6βH-eudesma-3-en-12,6α-olide (**4**): colourless gum; $[\alpha]_D^{20}$+57 (*c* 0.02, MeOH); HRESIMS *m/z* 405.1668 (calcd. for $C_{23}H_{26}O_5Na^+$, $[M-H_2O+Na]^+$ 405.1672); UV (MeOH) λ_{max} ($\log\varepsilon$) 225.2 (3.89), 278.2 (3.22) nm; IR (KBr) ν_{max} 3449, 1759, 1717, 1618, 1517, 1456 cm^{-1}; ^1H NMR (D_2O, 400 MHz): δ 5.60 (1H, br s, H-3), 4.39 (1H, d, *J* = 11.6 Hz, H-15a), 4.17 (1H, d, *J* = 11.6 Hz, H-15b), 4.07 (1H, dd, *J* = 8.8, 7.2 Hz, H-1), 2.94 (1H, t, *J* = 10.8 Hz, H-6), 2.51 (1H, m, H-2a), 2.06 (1H, m, H-2b), 2.00 (1H, d, *J* = 10.8 Hz, H-5), 1.88 (1H, m, H-8a), 1.80 (1H, m, H-11), 1.67 (1H, m, H-9a), 1.21 (1H, m, H-7), 1.08 (1H, m, H-8b), 1.05 (1H, m, H-9b), 0.86 (3H, d, *J* = 6.8 Hz, H-13), 0.58 (3H, s, H-14), R_1: 6.91 (2H, d, *J* = 8.4 Hz, H-2′ and H-6′), 6.61 (2H, d, *J* = 8.4 Hz, H-3′ and H-5′), 3.37 (1H, d, *J* = 14.8 Hz, H-7′a), 3.29 (1H, d, *J* = 14.8 Hz, H-7′b); ^{13}C NMR (D_2O, 100 MHz): δ 183.6 (C, C-12), 130.8 (C, C-4), 130.5 (CH, C-3), 82.8 (CH, C-1), 81.7 (CH, C-6), 68.7 (CH_2, C-15), 52.1 (CH, C-7), 48.1 (CH, C-5), 40.6 (CH, C-11), 39.8 (C, C-10), 34.4 (CH_2, C-9), 30.4 (CH_2, C-2), 22.0 (CH_2, C-8), 11.5 (CH_3, C-13), 11.3 (CH_3, C-14), R_1: 174.0 (C, C-8′), 154.9

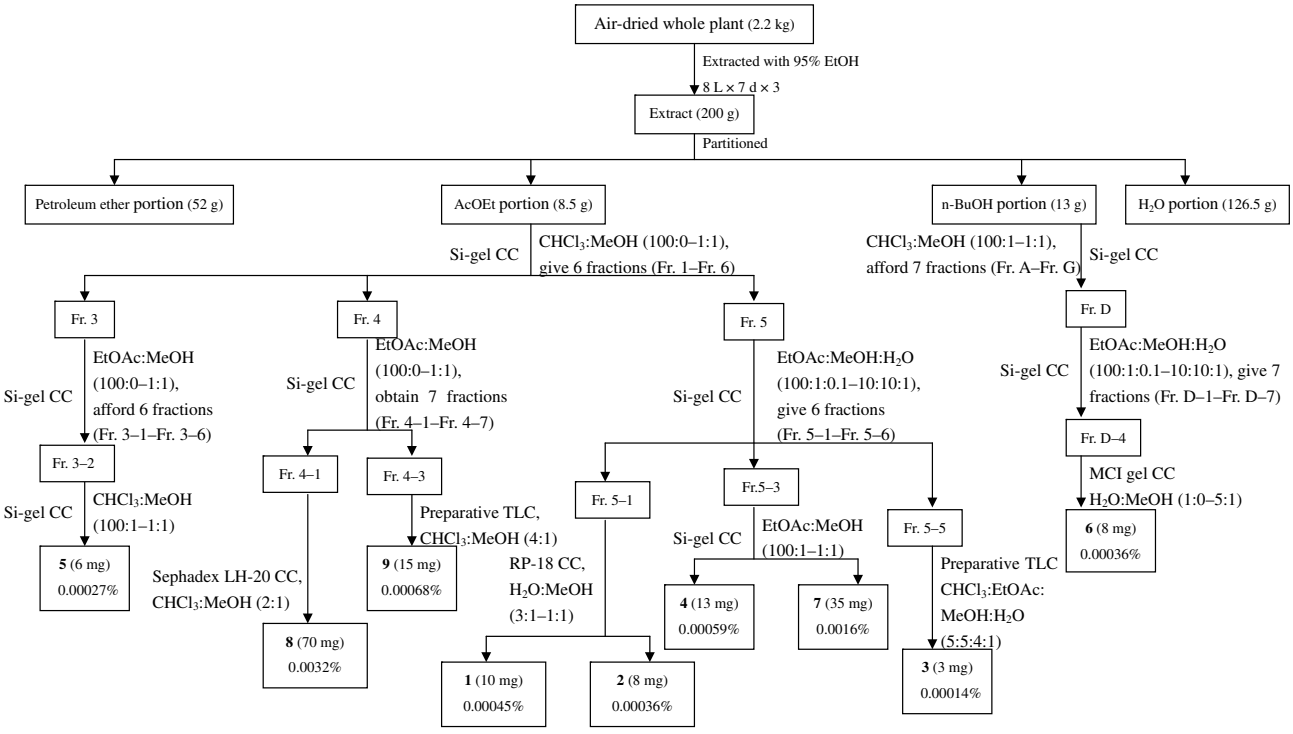


Fig. 1. Purification scheme of the isolated compounds.

Table 1
^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1**–**3** (400 MHz)

Position	**1**[a]		**2**[b]		**3**[c]	
	δ_C (mult.)	δ_H (mult., *J*)	δ_C (mult.)	δ_H (mult., *J*)	δ_C (mult.)	δ_H (mult., *J*)
1	82.9 (s)		82.9 (s)		74.2 (s)	
2	33.3 (t)	2.59 (d, 15.6)/2.33 (d, 15.6)[d]	32.7 (t)	2.34 (t, 12.8)/2.08 (d, 16.4)	36.0 (t)	2.28 (m)/2.08 (m)
3	69.8 (d)	5.28 (br s)	69.3 (d)	5.21 (d, 3.2)	69.3 (d)	5.36 (m)
4	73.2 (d)	4.86 (dd, 10.0, 3.6)	76.0 (d)	4.56 (dd, 11.6, 3.2)	71.8 (d)	5.03 (dd, 8.4, 3.6)
5	68.1 (d)	5.33 (td, 12.0, 4.8)	64.5 (d)	3.93 (td, 11.6, 4.8)	68.5 (d)	5.43 (m)
6	38.4 (t)	2.33 (d, 12.0)[d]/1.81 (t, 12.0)	39.4 (t)	2.25 (d, 16.4)/1.67 (t, 12.8)	38.0 (t)	2.18 (m)/2.13 (m)
7	172.3 (s)		176.6 (s)		174.7 (s)	
R_1			R_1		R_2	
1′	124.8 (s)[d,e]		125.6 (s)[d,j]		125.9 (s)	
2′, 6′	130.9 (d)[f]	6.97 (d, 8.0)[d]	131.0 (d)[d]	6.90 (d, 8.4)	131.3 (d)	7.14 (d, 8.0)
3′, 5′	115.8 (d)[d, g]	6.65 (d, 8.0)[d,i]	115.7 (d)[d,k]	6.58 (d, 8.4)[m]	116.0 (d)	6.75 (d, 8.0)[n]
4′	157.0 (s)[d]		154.9 (s)[l]		157.2 (s)[d]	
7′	40.3 (t)[h]	3.26 (s)	40.4 (t)	3.35 (d, 16.4)/3.30 d (16.4)	40.8 (t)	3.49 (d, 14.8)/3.43 d (14.8)
8′	171.7 (s)		173.6 (s)		171.7 (s)	
R_2			R_2		R_3	
1″	125.4 (s)[e]		125.7 (s)[d,j]		125.7 (s)[d]	
2″, 6″	131.1 (d)[f]	7.06 (d, 8.4)	131.1 (d)	6.74 (d, 8.0)	131.2 (d)	7.01 (d, 8.4)
3″, 5″	115.6 (d)[d,3]	6.63 (d, 8.4)[i]	115.7 (d)[d,k]	6.57 (d, 8.0)[m]	116.0 (d)[d]	6.78 (d, 8.4)[n]
4″	156.7 (s)		155.0 (s) [d,l]		157.2 (s) [d]	
7″	40.9 (t)	3.45 (s)	40.0 (t)	2.99 (d, 15.6)/2.93 (d, 15.6)	40.5 (t)	3.27 (s)
8″	170.8 (s)		174.0 (s)		171.4 (s)	
R_3			R_3		R_4	
1‴	124.8 (s)[d]		125.6 (s)[d,j]		125.7 (s)[d]	
2‴, 6‴	130.7 (d)[f]	6.83 (d, 8.4)	131.0 (d)[d]	6.72 (d, 8.8)	131.1 (d)	7.07 (d, 8.0)
3‴, 5‴	115.9 (d)[d,g]	6.67 (d, 8.4)[i]	115.7 (d)[d,l]	6.56 (d, 8.8)[m]	116.0 (d)[d]	6.77 (d, 8.0)[n]
4‴	157.0 (s)[d]		155.0 (s)[d,l]		157.2 (s)[d]	
7‴	39.8 (t)[h]	3.38 (s)	39.6 (t)	3.00 (d, 16.0)/3.14 (d, 16.0)	40.6 (t)	3.38 (s)
8‴	171.2 (s)		174.2 (s)		171.2 (s)	
R_4						
1⁗	124.6 (s)[e]					
2⁗, 6⁗	130.8 (d)[f]	6.97 (d, 8.0)[d]				
3⁗, 5⁗	115.8 (d)[g]	6.65 (d, 8.0)[d,i]				
4⁗	157.0 (s)					
7⁗	40.1 (t)[h]	3.00 (s)				
8⁗	171.3 (s)					
OMe					52.7 (q)	3.69 (s)

[a] Spectra were measured in DMSO-d_6.
[b] Spectra were measured in D_2O.
[c] Spectra were measured in acetone-d_6.
[d] Overlapping signals.
[e–n] Assignments may be reversed.

(C, C-4′), 131.3 (2CH, C-2′ and C-6′), 126.1 (C, C-1′), 115.7 (2CH, C-3′ and C-5′), 40.9 (CH_2, C-7′).

1β-*O*-β-D-Glucopyranosyl-(6′-*O*-*p*-methoxyphenylacetyl)-15-*O*-(*p*-hydroxyphenylacetyl)-5α,6βH-eudesma-3,11(13)-dien-12,6α-olide (**5**): colourless gum; $[\alpha]_D^{20}$+48 (*c* 0.02, MeOH); HRESIMS *m/z* 726.3130 (calcd. for $C_{38}H_{48}NO_{13}^+$, $[M+NH_4]^+$ 726.3120); UV (MeOH) λ_{max} (log ε) 226.2 (4.21), 276.6 (3.53) nm; IR (KBr) ν_{max} 3406, 1740, 1616, 1515, 1457 cm^{-1}; ^{1}H NMR (acetone-d_6, 300 MHz): δ 5.92 (1H, d, *J* = 3.0 Hz, H-13a), 5.60 (1H, br s, H-3), 5.42 (1H, d, *J* = 3.0 Hz, H-13b), 4.68 (1H, m, H-5′), 4.57 (1H, d, *J* = 12.3 Hz, H-15a), 4.50 (1H, d, *J* = 7.5 Hz, H-1′), 4.43 (1H, d, *J* = 12.3 Hz, H-15b), 3.85 (1H, dd, *J* = 12.0, 2.4 Hz, H-6′a), 3.70 (1H, dd, *J* = 10.2, 7.2 Hz, H-1), 3.66 (1H, m, H-2′), 3.47 (1H, t, *J* = 11.1 Hz, H-6), 3.40 (1H, dd, *J* = 12.0, 5.7 Hz, H-6′b), 3.35 (1H, m, H-4′), 3.29 (1H, m, H-3′), 2.38 (1H, m, H-2a), 2.35 (1H, m, H-7), 2.32 (1H, d, *J* = 11.1 Hz, H-5), 1.96 (1H, m, H-9a), 1.81 (1H, m, H-8a), 1.47 (1H, m, H-2b), 1.39 (1H, m, H-8b), 1.28 (1H, m, H-9b), 0.55 (3H, s, H-14), R_1: 7.24 (2H, d, *J* = 8.1 Hz, H-2″ and H-6″), 6.87 (2H, d, *J* = 8.1 Hz, H-3″ and H-5″), 3.50 (2H, s, H-7″), R_2: 7.04 (2H, d, *J* = 8.1 Hz, H-2‴ and H-6‴), 6.73 (2H, d, *J* = 8.1 Hz, H-3‴ and H-5‴), 3.60 (2H, s, H-7‴), 3.78 (3H, s, OMe); ^{13}C NMR (acetone-d_6, 75 MHz): δ 170.6 (C, C-12), 140.4 (C, C-11), 132.5 (C, C-4), 128.2 (CH, C-3), 116.5 (CH_2, C-13), 98.3 (CH, C-1′), 80.9 (CH, C-6), 79.3 (CH, C-1), 77.5 (CH, C-5′), 75.6 (CH, C-3′), 74.9 (CH, C-2′), 71.9 (CH, C-4′), 67.7 (CH_2, C-15), 62.7 (CH_2, C-6′), 51.2 (CH, C-7), 49.9 (CH, C-5), 40.5 (C, C-10), 35.2 (CH_2, C-9), 29.2 (CH_2, C-2), 21.5 (CH_2, C-8), 12.2 (CH_3, C-14), R_1: 171.4 (C, C-8″), 157.1 (C, C-4″), 131.4 (2CH, C-2″ and C-6″), 116.1 (2CH, C-3″ and 5″), 126.1 (C, C-1″), 41.2 (CH_2, C-7″), R_2: 171.0 (C, C-8‴), 159.7 (C, C-4‴), 131.4 (2CH, C-2‴ and C-6‴), 114.6 (2CH, C-3‴ and C-5‴), 127.3 (C, C-1‴), 41.1 (CH_2, C-7‴), 55.5 (CH_3, OMe).

3. Results and discussion

3.1. Phytochemical investigation

The EtOH extract from the whole plant of *S. arvensis* was subjected to liquid–liquid fractionation, and the EtOAc and *n*-BuOH-soluble fractions were repeatedly separated by column chromatography (silica gel, Sephadex LH-20, RP-18 silica gel, and MCI gel) to yield three new (*p*-hydroxyphenylacetyl)quinic acids (**1**–**3**), two new (**4** and **5**) and four known eudesmanolides (**6**–**9**).

Compound **1**, a yellow gum, has the molecular formula of $C_{39}H_{36}O_{14}$, deduced from its HRESIMS (*m/z* 727.2022, $[M-H]^-$). Hydroxyl groups are indicated by a band at 3423 cm^{-1}, ester carbonyls at 1722 cm^{-1}, and benzyl groups by bands at 1615, 1516, and 1446 cm^{-1}. The ^{1}H NMR (Table 1) spectrum of **1** displays characteristic peaks of four methylenes at 3.26 (s), 3.45 (s), 3.38 (s), and 3.00 (s). In the region of aromatic protons, signals of four A_2B_2 systems [δ_H 6.97 (2H, d, *J* = 8.0 Hz, H-2′, 6′), 6.65 (2H, d, *J* = 8.0 Hz, H-3′,

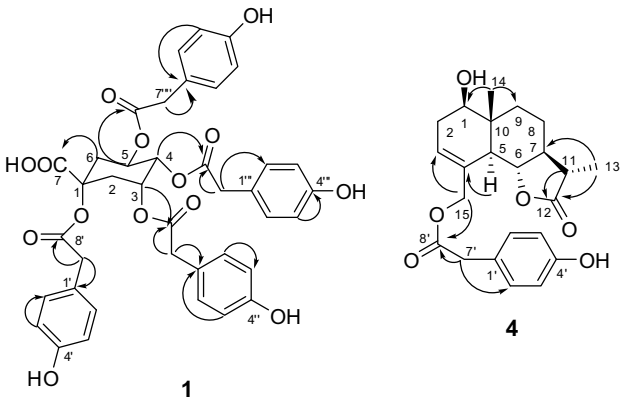
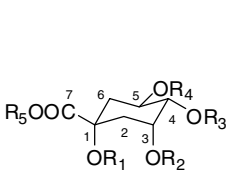


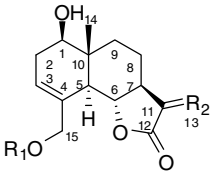
Fig. 2. Key HMBC correlations of compound **1** and **4**.

5′); δ_H 7.06 (2H, d, J = 8.4 Hz, H-2″, 6″), 6.63 (2H, d, J = 8.4 Hz, H-3″, 5″); δ_H 6.83 (2H, d, J = 8.4 Hz, H-2‴, 6‴), 6.67 (2H, d, J = 8.4 Hz, H-3‴, 5‴); δ_H 6.97 (2H, d, J = 8.0 Hz, H-2⁗, 6⁗), 6.65 (2H, d, J = 8.0 Hz, H-3⁗, 5⁗)] were observed and assigned to protons on four 1,4-disubstituted benzene rings. In conjunction with ^{13}C NMR spectroscopic data, four *p*-hydroxyphenylacetyl moieties were inferred to be present in **1** (Han, Zhang, Gao, & Jia, 2005) (Table 1).

The quinic acid moiety was assigned by ^1H NMR peaks at δ_H 5.28, 4.86, and 5.33 for three oxymethines, at δ_H 2.333/2.59 for two pairs of sp^3 methylenes and at 1.81/2.335 for H-2 and H-6, respectively (Table 1). By analysis of ^{13}C NMR spectra, peaks at δ_C 69.8, 73.2, and 68.1 are assigned to three oxymethines, δ_C 33.3 and 38.4 to two sp^3 methylenes, δ_C 82.9 to an oxygenated quaternary carbon, and δ_C 172.3 to a carboxyl. All the carbons are characteristic of a quinic acid unit (Fumihiro et al., 2005; Kim et al., 2006). Assignments to the quinic acid nucleus were further corroborated by analysis of ^1H–^1H COSY and HMBC spectra of **1** (Fig. 2). Besides, H-3, H-4, and H-5 of the quinic acid moiety are assigned by their splitting patterns and spin–spin coupling constants (Morishita, Iwahashi, Osaka, & Kido, 1984). The location of *p*-hydroxyphenylacetyl substitutions in the quinic acid moiety are deduced from the comparative analysis of ^1H chemical shifts. That three oxymethine protons at H-3 (δ_H 5.28), H-4 (δ_H 4.86) and H-5 (δ_H 5.33) experience a deshielding effect indicates the acylation of hydroxyl groups at these positions, as reported previously for other natural quinic acid derivatives (Cheminat, Zawatzky, Becker, & Brouillard, 1988; Pauli, Kuczkowiak, & Nahrstedt, 1999; Pauli, Poetsch, & Nahrstedt, 1998). These assignments are further supported by the analysis of HMBC spectra (Fig. 2). Comparison of ^{13}C NMR shifts observed for C-1 (δ_C 82.9) with those reported for 3,4,5-trigalloylquinic acid (δ_C 74.7) (Altmann & Falk, 1995) and 1,3,4,5-tetragallylquinic acid (δ_C 80.6) (Altmann & Falk, 1995) verifies the assumption that the OH group at C-1 is acylated, previously reported for other 1-acylquinic acid derivatives, such as δ_C 80.7 for C-1 of 1,4,5-tri-*O*-caffeoylquinic acid (Merfort, 1992; Wenzl, Chaves, Mayer, Rao, & Nair, 2000). From these results, compound **1** is inferred to be 1,3,4,5-tetra-(*p*-hydroxyphenylacetyl)quinic acid (Fig. 3).

Compound **2** was obtained as a colourless gum. Its molecular formula, $C_{31}H_{30}O_{12}$, was established by HRESIMS (m/z 617.1622, $[M+Na]^+$). Its ^1H and ^{13}C NMR data show that **2** consists of three *p*-hydroxyphenylacetate moieties and a quinic acid moiety (Table 1). ^1H NMR data for **2** (Table 1) manifest H-3 (δ_H 5.21) and H-4 (δ_H 4.56) of two CHOHs on the cyclohexane ring assigned as acylated. They experience a deshielding effect. But, H-5 (δ_H 3.93) of one CHOH group on the cyclohexane ring is unsubstituted (Agata, Goto, Hatano, Nishibe, & Okuda, 1993). In the HMBC experiment, the cross-peaks observed between H-3 (δ_H 5.21) and C-8″ (δ_C 174.0), as well as those between H-4 (δ_H 4.56) and C-8‴ (δ_C 174.2), indicate that two substitutions in *p*-hydroxyphenylacetate moieties are attached to C-3 and C-4, respectively, and the third *p*-hydroxyphenylacetyl group is attached to C-1, whose ^{13}C NMR peak (δ_C





1 $R_1 = R_2 = R_3 = R_4 = A \quad R_5 = H$

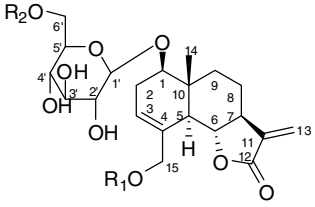
2 $R_1 = R_2 = R_3 = A \quad R_4 = R_5 = H$

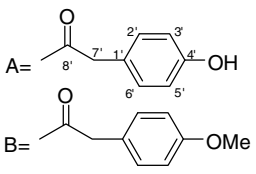
3 $R_1 = H \quad R_2 = R_3 = R_4 = A \quad R_5 = Me$

4 $R_1 = A \quad R_2 = \alpha\text{-Me}, \beta\text{-H}$

6 $R_1 = H \quad R_2 = \alpha\text{-Me}, \beta\text{-H}$

7 $R_1 = A \quad R_2 = CH_2$





5 $R_1 = A \quad R_2 = B$

8 $R_1 = A \quad R_2 = H$

9 $R_1 = R_2 = A$

Fig. 3. The structure of compounds **1**–**9**.

82.9) shifts downfield. As such, compound **2** is assumed to be 1,3,4-tri-(*p*-hydroxyphenylacetyl)quinic acid (Fig. 3).

Compound **3** appeared as a yellow gum. Its structure was characterized by spectroscopic data (Table 1). Its molecular formula, $C_{32}H_{32}O_{12}$, is deduced from HRESIMS determination (*m/z* 631.1781, $[M+Na]^+$). ^1H and ^{13}C NMR data for **3** (Table 1) show it to consist of a quinic acid and three *p*-hydroxyphenylacetyl groups attached to positions 3, 4, and 5 of the quinic residue, where the ^1H NMR peak of H-3 appears at 5.36, H-4 at 5.03, and H-5 at 5.43, respectively (Bouchet, Levesque, Bodo, & Pousset, 1998). NMR peaks, at δ_H 3.69 and δ_C 52.7 for the methyl group, enable us to assign it unequivocally as connected to an ester carbonyl rather than an aromatic ether because a long-range correlation was observed in HMBC experiments between the proton of the methyl group and the carbonyl carbon at 174.7 (C-7). Consequently, compound **3** was assumed to 3,4,5-tri-(*p*-hydroxyphenylacetyl)quinic acid methyl ester (Fig. 3).

Compound **4** was obtained as a colourless gum. Its molecular formula, $C_{23}H_{28}O_6$, is deduced from HRESIMS measurement (*m/z* 405.1668, $[M-H_2O+Na]^+$). Hydroxyl groups are indicated by a band at 3449 cm^{-1}, γ-lactone group at 1759 cm^{-1}, ester carbonyl at 1717 cm^{-1}, and benzyl groups by bands at 1618, 1517, 1456 cm^{-1}. ^1H, ^{13}C NMR, and DEPT spectra reveal that **4** contains a *p*-hydroxyphenylacetate moiety. Except for a ^{13}C signal assigned to the *p*-hydroxyphenylacetate moiety, the ^{13}C NMR spectrum of **4** exhibits 15 skeleton carbons, which are two CH_3, four CH_2, six CH, and three quaternary carbons. By the comparison of NMR data of **4** to those of known compounds (Han et al., 2005; Zhang, Xie, Li, Shi, & Jia, 2006), compound **4** is assumed to be (1β,6α)-1,6-dihydroxy-14-*O*-[(4-hydroxyphenyl)acetyl]eudesma-3,11(13)-dien-12-oic acid γ-lactone (**7**). The main difference in the structures of **4** and **7** is that the methylene-γ-lactone group in **7** is replaced by a methyl-γ-lactone group in **4**. The *p*-hydroxyphenylacetate group linked at C-15 is deduced from HMBC correlation between H-15 (δ_H 4.39, 4.17) and C-8′ (δ_C 174.0) in **4** (Fig. 2). The stereochemistry is inferred from coupling constants and NOE experiments. The large coupling constants observed for H-1 [δ_H 4.07 (1H, dd, J = 8.8, 7.2 Hz)] with H-2 ($J_{1\alpha,2\beta}$ = 8.8 Hz), and H-6 [δ_H 2.94 (1H, t, J = 10.8 Hz) with H-5 ($J_{6\beta,5\alpha}$ = 10.8 Hz) and H-7 ($J_{6\beta,7\alpha}$ = 10.8 Hz) indicate that the stereochemistry of H-1 favours an α-orientation and that the lactone group at C-6 and C-7 has a *trans* (6β,7α) stereochemical relationship. NOE difference spectra of **4** show that irradiation of H-14 enhances H-6 and irradiation of H-6 enhances H-11 and H-14. Thus, the stereochemistry of H-6, H-11 and H-13 favours a β-orientation. The fact that irradiation of H-7 enhances H-5 and H-13 indicates that the stereochemistry of H-5, H-7 and H-13 favours an α-orientation. As such, compound **4** was inferred to be 1β-hydroxy-15-*O*-(*p*-hydroxyphenylacetyl)-5α,6βH-eudesma-3-en-12,6α-olide (Fig. 3).

Compound **5**, a colourless gum, has a molecular formula, $C_{38}H_{44}O_{13}$, deduced from HRESIMS (*m/z* 726.3130, $[M+NH_4]^+$). Its hydroxyl groups are indicated by a band at 3406 cm^{-1}, α-methylene-γ-lactone group at 1740 cm^{-1}, and benzyl groups by bands at 1616, 1515, 1457 cm^{-1}. ^1H, ^{13}C NMR, and DEPT spectra allow us to assign **5** as containing two *p*-hydroxyphenylacetate moieties. Typical NMR peaks of β-glucopyranoside are readily recognized from NMR data (Yang, Shi, & Jia, 2002). In addition, NMR data of **5** show the existence of a methylene-γ-lactone group [δ_H 5.92 (1H, d, J = 3.0 Hz, H-13a), 5.42 (1H, d, J = 3.0 Hz, H-13b); δ_C 140.4 (qC, C-11), 170.6 (qC, C-12), 116.5 (CH_2, C-13)] and a methoxyl group [δ_H 3.78 (3H, s); δ_C 55.5 (CH_3)]. These two *p*-hydroxyphenylacetate moieties are attached to C-15 and C-6′, confirmed by HMBC correlations between H-15 (δ_H 4.57, 4.43) and an ester carbonyl C-8″ (δ_C 171.4) as well as between H-6′ (δ_H 3.85, 3.40) and the other ester carbonyl C-8‴ (δ_C 171.0). HMBC cross-peaks between H-1 (δ_H 3.70) and C-1′ (δ_C 98.3), as well as H-1′ (δ_H 4.50)] and C-1 (δ_C 79.3), allow us to assume that the β-glucopyranosyl group is linked at C-1. The coupling constant of H-1′ (J = 7.5 Hz) suggests that glucose lies along the β-orientation. D-glucose is confirmed by PC and its optical rotation (α_D^{20}+46 (c 0.01, H_2O)). The optical rotation measurement was run on the species of **5** hydrolyzed with HCl. By the comparison of spectroscopic data with those for known compounds (Han et al., 2005; Zhang et al., 2006), the structure of **5** seems to be very similar to 1β-*O*-β-D-glucopyranosyl-(6′-*O*-*p*-hydroxyl-phenylacetyl)-15-*O*-(*p*-hydroxyphenylacetyl)-5α,6βH-eudesma-3,11(13)-dien-12, 6α-olide (**9**), except for a methoxyl group (δ_H 3.78; δ_C 55.5). The linkage of the methoxyl group at C-4‴ is deduced from HMBC correlation between the methoxyl group (δ_H 3.78) and C-4‴ (δ_C 159.7). Large coupling constants for H-1 with H-2 ($J_{1\alpha,2\beta}$ = 10.2 Hz) and H-6 with H-5 ($J_{6\beta,5\alpha}$ = 11.1 Hz) and H-7 ($J_{6\beta,7\alpha}$ = 11.1 Hz) indicate that H-1 lies along the α-orientation and the lactone group is attached to C-6 and C-7 in a *trans* (6β,7α) relationship. The NOE difference spectrum shows that irradiation of H-14 enhances H-6, indicating H-6 lying along the β-orientation. Thus, compound **5** is inferred to be 1β-*O*-β-D-glucopyranosyl–(6′-*O*-*p*-methoxyphenylacetyl)-15-*O*-(*p*-hydroxyphenylacetyl)-5α,6βH-eudesma-3,11(13)-dien-12,6α-olide (Fig. 3).

The known compounds were identified as (1β,6α)-1,6,14-trihydroxyeudesm-3-en-12-oic acid γ-lactone (**6**) (Zhang et al., 2006), (1β,6α)-1,6-dihydroxy-14-O-[(4-hydroxyphenyl) acetyl]eudesma-3,11(13)-dien-12-oic acid γ-lactone (**7**) (Zhang et al., 2006), 1β-*O*-β-D-glucopyranosyl-or-15-*O*-(*p*-hydroxyphenylacetyl)-5α,6βH-eudesma-3,11(13)-dien-12,6α-olide (**8**) (Han et al., 2005), and 1β-*O*-β-D-glucopyranosyl-(6′-*O*-*p*-hydroxyphenylacetyl)-15-*O*-(*p*-hydroxyphenylacetyl)-5α,6βH-eudesma-3,11(13)-dien-12,6α-olide (**9**) (Han et al., 2005) (Fig. 3) by comparing their physical and spectroscopic data with those reported in the literatures.

In the present study, the three quinic acid derivatives (**1**–**3**), and two new eudesmanolides (**4** and **5**) are newly isolated from *S. arvensis*. It is particularly noteworthy that the *p*-hydroxyphenylacetyl moiety in **1**–**3** rarely appears in any other quinic acid derivatives reported to date [only one analogue of 3,5-di-*O*-caffeoyl-4-*O*-[(4-hydroxyphenyl)acetyl]quinic acid (Sprogøe et al., 2007)]. Compounds **1**–**3** are considered to be representative of a whole new class of naturally occurring polyphenolic compounds, (*p*-hydroxyphenylacetyl)quinic acid. They will enter into the architectural diversity of the quinic acid family. The four known eudesmanolides (**6**–**9**) are found for the first time in this plant.

3.2. DPPH free radical-scavenging activity

Free radical-scavenging activities of compounds **1**–**9** were evaluated by comparison with those of known antioxidants (caffeic acid and ascorbic acid) using DPPH· (Kang et al., 2007). A methanolic solution of each of the compounds **1**–**9** was mixed with a methanolic solution of DPPH·. The final DPPH· concentration was 40 mg l^{-1}. The final concentrations of probe were 5, 10, 20, 40, 80, and 160 μg/ml, respectively. After incubation in 96-well plates, the mixture (250 μl) was kept in the dark at ambient temperature (25 °C) for 30 min. The optical density of the mixture was determined in comparison with DPPH· and pure methanol on a Bio-RAD Benchmark Plus Plate Reader at 517 nm. Each value given was an average of at least three measurements. The scavenging ability of antioxidant was calculated according to:

$$\text{DPPH·-scavenging activity } (\%) = (A_0 - A)/A_0 \times 100$$

where A_0 is the absorbance of the control reaction and A the absorbance in the presence of samples. IC_{50} value is negatively related to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease the radical concentration by 50%. The lower

the IC_{50} value, the higher is the antioxidant activity of the tested sample. Unfortunately, all these compounds showed no antioxidant activity.

4. Conclusion

DPPH radical is commonly used as a substrate to evaluate antioxidant activity; it is a stable free radical that can accept an electron or hydrogen radical to become a stable molecule. Polyphenols are secondary plant metabolites that have been reported to have anti-carcinogenic, anti-mutagenic and antioxidant activities. They can act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evas, Miller, & Paganga, 1996). The DPPH radical-scavenging capability of all the isolated compounds was investigated, but none showed antioxidant activity. The absence of antioxidant activity could be caused by the absence of *ortho* or *para*-diphenolic groups in all detected compounds, that are responsible of the activity against free radicals by an electron transfer reaction, as in $DPPH^{\cdot}$-scavenging assay (Kajiyama & Ohkatsu, 2001).

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