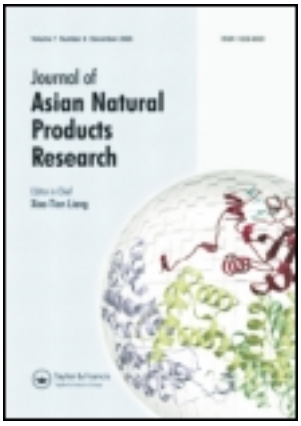

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
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Available online: 10 Aug 2011

To cite this article: Wen-Jing Wang, Ying Wang, Qin-Wen Zhang, Xiao-Qi Zhang, Ting-Ting Yang, Yi Dai, Lei Wang, Lei-Hong Zhang & Wen-Cai Ye (2011): Chemical constituents from *Eupatorium chinense* , Journal of Asian Natural Products Research, 13:9, 845-850

To link to this article: http://dx.doi.org/10.1080/10286020.2011.598149

Journal of Asian Natural Products Research
Vol. 13, No. 9, September 2011, 845–850

Chemical constituents from *Eupatorium chinense*

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(*Received 18 April 2011; final version received 14 June 2011*)

Two new monoterpenoid glycosides, α-(*E*)-acaridiol 8-*O*-β-D-glucopyranoside (**1**) and α-(*E*)-acaridiol 9-*O*-β-D-glucopyranoside (**2**), as well as a new thiophen, 2-acetyl-3-hydroxy-5-(prop-1-ynyl)thiophen 3-*O*-β-D-glucopyranoside (**3**), were isolated from the roots of *Eupatorium chinense*. Their structures were determined on the basis of extensive spectroscopic and chemical analyses.

Keywords: *Eupatorium chinense*; monoterpenoid glycoside; thiophen

1. Introduction

The plants of genus *Eupatorium* (Compositae) are mainly spread in the temperate areas of USA and East Asia. Previous phytochemical investigation revealed that the main constituents in the plants of this genus were sesquiterpene lactones [1–5], triterpenoids [6,7], flavonoids [5,6], benzofurans [7,8], and lignans [7]. The dried root of *Eupatorium chinense* had been widely used as a Chinese folk medicine for the treatment of multifarious throat disorders for a long time. The potential medicinal importance of *E. chinense* prompted us to initiate a phytochemical study on the roots of this plant, which led to the isolation of two new monoterpenoid glycosides, α-(*E*)-acaridiol 8-*O*-β-D-glucopyranoside (**1**) and, α-(*E*)-acaridiol 9-*O*-β-D-glucopyranoside (**2**), and a new thiophen, 2-acetyl-3-hydroxy-5-(prop-1-ynyl)thiophen 3-*O*-β-D-glucopyranoside (**3**), along with five known compounds (**4**–**8**) (Figure 1). In this paper, the isolation and structural elucidation of the two new isomeric monoterpenoid glycosides and the new thiophen are described.

2. Results and discussion

Compound **1** was obtained as a colorless gum. The molecular formula of **1** was established as $C_{16}H_{28}O_7$ by HR-ESI-MS at *m/z* 355.1730 $[M + Na]^+$. The ^1H NMR spectrum of **1** showed signals for two vinyl protons [δ_H 5.63 and 5.12 (each 1H, m)], two oxo-methylenes [δ_H 4.40 (1H, m), 4.29 (1H, m) and 4.00 (2H, br s)], two methylenes [δ_H 2.14 (2H, m) and 2.10 (2H, m)], two methyls [δ_H 1.67 and 1.60 (each 3H, s)], and an anomeric proton [δ 4.28 (1H, d, $J = 7.8$ Hz)]. The ^{13}C NMR and DEPT spectra of **1** exhibited 16 carbon signals including 4 olefinic carbons, 4 methylenes, 2 methyls and a sugar unit. With the aid of ^1H–^1H COSY, HSQC, HMBC, and NOESY experiments, the

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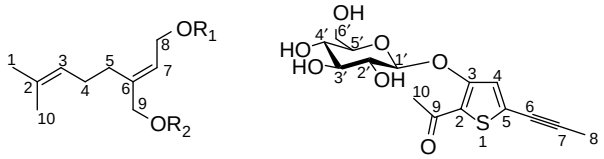
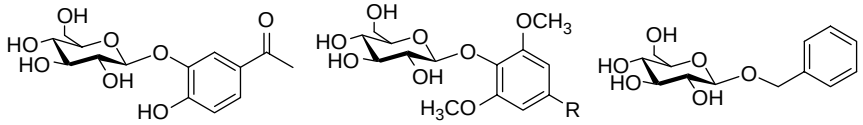
ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.598149
http://www.informaworld.com



1 R_1 = Glc, R_2 = H
2 R_1 = H, R_2 = Glc

3



4

5 R = OH
6 R = COOH
7 R = CH_2OH

8

Figure 1. Chemical structures of compounds **1**–**8**.

NMR signals of **1** were assigned as shown in Table 1.

The above ^1H and ^{13}C NMR signals of the aglycone and sugar unit of **1** were similar to those of the known compound α-(*E*)-acaridiol [9] and glucopyranosyl [10]. The HMBC correlations between the proton at δ_H 5.12 (H-3) and the carbons at δ_C 25.9 (C-1)/17.8 (C-10), and between the proton at δ_H 5.63 (H-7) and the carbons at δ_C 29.2 (C-5)/66.3 (C-9), confirmed the planar structure of the aglycone of **1**

Table 1. ^1H and ^{13}C NMR spectral data of compounds **1** and **2** (CD_3OD, δ in ppm, J in Hz)[a].

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
1	25.9 q	1.67 s	25.9 q	1.67 s
2	133.0 s	–	132.8 s	–
3	125.0 d	5.12 m	125.1 d	5.12 m
4	28.2 t	2.10 m	28.0 t	2.10 m
5	29.2 t	2.14 m	29.1 t	2.14 m
6	144.4 s	–	139.3 s	–
7	122.5 d	5.63 m	128.7 d	5.68 t (6.6)
8	66.2 t	4.40 m 4.29	59.0 t	4.13 dd (6.6, 2.8)
9	66.3 t	4.00 br s	73.2 t	4.30 d (12.3) 4.08 d (12.3)
10	17.8 q	1.60 s	17.8 q	1.61 s
1′	103.1 d	4.28 d (7.8)	103.1 d	4.27 d (7.8)
2′	75.1 d	3.19	75.1 d	3.19
3′	77.9 d	3.25	77.9 d	3.25
4′	71.7 d	3.28	71.8 d	3.28
5′	78.2 d	3.33	78.2 d	3.33
6′	62.7 t	3.85 dd (12.0, 2.2) 3.66 dd (12.0, 5.5)	62.8 t	3.86 dd (12.0, 2.2) 3.65 dd (12.0, 5.4)

Note: [a] Overlapped signals were reported without designating multiplicity.

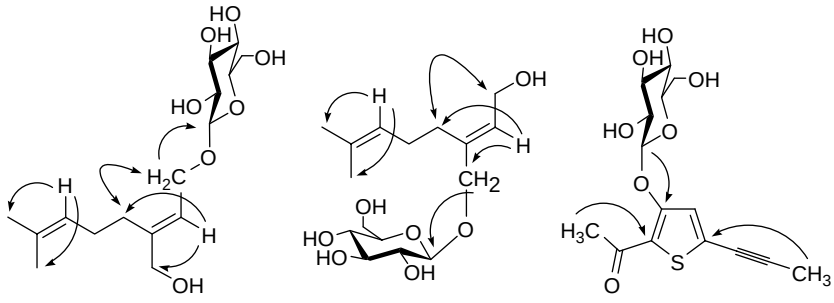


Figure 2. Key HMBC (→) and NOESY (↔) correlations of **1**–**3**.

(Figure 2). The NOESY correlation between H-5 at δ_H 2.14 and H-8a at δ_H 4.40 suggested that the geometry of the double bond was *E* form. Furthermore, the HMBC correlation between H-8a at δ_H 4.40 and C-1′ at δ_C 103.1 indicated that the glucopyranosyl unit was located at C-8. Thus, the structure of **1** was elucidated as α-(*E*)-acaridiol 8-*O*-β-D-glucopyranoside.

Compound **2** was obtained as a colorless gum. Compound **2** showed the same molecular formula as **1** by its HR-ESI-MS. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **2** were also very similar to those of **1**, except that the C-9 resonance in **2** exhibited a comparative downfield shift, whereas the C-8 resonance displayed somewhat upfield shift. Analysis of the ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY spectral data of **1** and **2** led to the conclusion that the two compounds were isomers due to the different location of the sugar unit. The HMBC correlation between H-9b at δ_H 4.08 and C-1′ at δ_C 103.1 suggested that the monosaccharide moiety was located at C-9. The NOESY correlation between H-5 at δ_H 2.14 and H-8 at δ_H 4.13 confirmed that the geometry of the double bond was also *E* form. Thus, the structure of **2** was elucidated as α-(*E*)-acaridiol 9-*O*-β-D-glucopyranoside.

Compound **3** was obtained as an amorphous powder. The quasi-molecular ion at *m*/*z* 365.0665 $[M + Na]^+$ in its HR-ESI-MS indicated that the molecular formula of **3** was $C_{15}H_{18}O_7S$. The IR spectrum of **3** suggested the presence of hydroxyl group (3299 cm^{-1}), acetylenic bond (2234 cm^{-1}), and carbonyl group (1624 cm^{-1}). The UV spectrum showed absorption maxima at 226 and 316 nm. The above data suggested that **3** may be a thiophen. The ^{1}H NMR spectral data of **3** showed the presence of an olefinic proton [δ_H 7.08 (1H, s)], two methyls [δ_H 2.56 and 2.07 (each 3H, s)], and an anomeric proton [δ_H 5.01 (1H, d, $J = 7.4$ Hz)]. The ^{13}C NMR and DEPT spectra of **3** showed 15 carbon signals including a carbonyl, 4 olefinic carbons, 2 methyls, and a sugar unit. Acid hydrolysis of **3** suggested the presence of D-glucopyranosyl residue. The HMBC correlations between H-10 at δ_H 2.56 and C-2 at δ_C 125.1, between H-1′ at δ_H 5.01 and C-3 at δ_C 158.4, as well as between H-8 at δ_H 2.07 and C-5 at δ_C 131.9 indicated that the acetyl, glucopyranosyl unit and prop-1-ynyl group were connected to C-2, C-3, and C-5 positions, respectively. Thus, the structure of **3** was characterized as 2-acetyl-3-hydroxy-5-(prop-1-ynyl)thiophen 3-*O*-β-D-glucopyranoside.

Other known compounds were identified as pungenin (**4**) [10], 2,6-dimethoxy-4-hydroxyphenol-*O*-β-D-glucoside (**5**) [11], syringic acid 4-*O*-β-D-glucopyranoside (**6**) [12], di-*O*-methylcrenatin (**7**) [11],

and benzyl-1-*O*-β-D-glucopyranoside (**8**) [13], by comparison of their physical and spectroscopic data with those reported previously.

3. Experimental

3.1 General experimental procedures

Melting points were measured on an X-5 micro melting point apparatus (Beijing Tech Instrument, Inc., Beijing, China). UV spectra were obtained on a Jasco V-550 UV/Vis spectrometer. IR spectra were obtained on a Jasco FT/IR-480 plus infrared spectrometer with KBr pellets. Optical rotations were determined on a Jasco P-1020 polarimeter. 1D and 2D NMR spectra were measured using a Bruker AV-400 spectrometer. HR-ESI-MS data were detected on an Agilent 6210 ESI/TOF mass spectrometer. Column chromatography (CC) was carried out on silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China), ODS (YMC, Kyoto, Japan), and Sephadex LH-20 (Pharmacia, NJ, USA), respectively. Preparative high-performance liquid chromatography (HPLC) was carried out on a Varian chromatograph equipped with a Prostar 215 pump, a Prostar 325 UV-vis detector, and a Cosmosil 5C_{18}-MS-II reversed-phase column (20 mm × 250 mm, 5.0 μm, Nacalai Tesque, Kyoto, Japan). Analytical HPLC was carried out on a Dionex chromatograph equipped with a P680 pump, a PDA-100 photodiode array detector, and a Cosmosil 5C_{18}-MS-II reversed-phase column (4.6 mm × 250 mm, 5.0 μm, Nacalai Tesque). Chiral separation was carried out on an Agilent 1200 system equipped with a G1315B DAD detector and a chiral CD-Ph column (4.6 mm × 250 mm, 5.0 μm, Shiseido Fine Chemicals Ltd, Tokyo, Japan).

3.2 Plant material

The roots of *E. chinense* were collected in Conghua city, Guangdong Province of China, in October of 2006, and authenticated by Prof. Shu-Yuan Li (Guangdong Pharmaceutical University). A voucher specimen (No. 2006101601) is deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, China.

3.3 Extraction and isolation

The dried and powdered roots of *E. chinense* (10 kg) were extracted with 70% (v/v) EtOH–H_2O (3 × 30 L, each 2 h) under reflux. The concentrated EtOH extract was suspended in H_2O (4 L) and then successively extracted with petroleum ether (4 × 4 L), EtOAc (4 × 4 L), and *n*-BuOH (4 × 4 L). The *n*-BuOH solution was concentrated to give a residue (180 g), which was subjected to silica gel column eluting with $CHCl_3$–MeOH (100:0 → 0:100) to afford seven fractions (Fr. A–G). Fr. C (3 g) was further purified using ODS column eluting with MeOH–H_2O (50:50 → 90:10) to give five fractions (Fr. C1—C5). Fr. C1 (183 mg) was subjected to preparative HPLC (MeOH–H_2O, 10:90; flow rate, 8 ml/min; wavelength, 210 nm) to yield **4** (4 mg), **5** (17 mg), and **7** (16 mg). Fr. C2 (50 mg) was subjected to preparative HPLC (MeOH–H_2O, 25:75; flow rate, 8 ml/min; wavelength, 210 nm) to obtain **8** (4 mg). Fr. C3 (246 mg) was separated by preparative HPLC (MeOH–H_2O, 40:60; flow rate, 8 ml/min; wavelength, 210 nm) to yield **3** (12 mg), **6** (7 mg), and a mixture of **1** and **2** (10 mg). The mixture was further separated into isomers **1** (0.5 mg, t_R 23.9 min) and **2** (0.3 mg, t_R 26.5 min) by chiral column chromatography (MeOH–H_2O, 20:80; column temperature, 30°C; flow rate, 0.8 ml/min; wavelength, 210 nm).

3.3.1 α-(E)-Acaridiol 8-O-β-D-glucopyranoside (1)

A colorless gum, IR ν_{max}^{KBr}: 3412, 3020, 2961, 1673, 1102, 1060, 810 cm^{-1}; ^1H

NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data, see Table 1; HR-ESI-MS *m/z* 355.1730 $[M + Na]^+$ (calcd for $C_{16}H_{28}O_7Na$, 355.1727).

3.3.2 α-(E)-Acaridiol 9-O-β-D-glucopyranoside (2)

A colorless gum, IR ν_{max}^{KBr}: 3413, 3019, 2961, 1673, 1107, 1062, 811 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data, see Table 1; HR-ESI-MS *m/z* 355.1731 $[M + Na]^+$ (calcd for $C_{16}H_{28}O_7Na$, 355.1727).

3.3.3 2-Acetyl-3-hydroxy-5-(prop-1-ynyl)thiophen 3-O-β-D-glucopyranoside (3)

An amorphous powder, mp 118–119°C; $[\alpha]_D^{25}$ −37.8 (*c* 0.7, CH_3OH); UV (MeOH) λ_{max} (log ε) 226 (4.11), 316 (4.50) nm; IR (KBr) ν_{max} 3299, 2234, 1624, 1454, 1100, 1080, 1041 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ: 7.08 (1H, s, H-4), 5.01 (1H, d, $J = 7.4$ Hz, H-1′), 3.88 (1H, dd, $J = 12.1$, 2.0 Hz, H-6′a), 3.69 (1H, dd, $J = 12.1$, 5.6 Hz, H-6′b), 3.47 (1H, m, H-2′), 3.46 (1H, m, H-3′), 3.43 (1H, m, H-5′), 3.39 (1H, m, H-4′), 2.56 (3H, s, H-10), 2.07 (3H, s, H-8); ^{13}C NMR (CD_3OD, 100 MHz) δ: 192.6 (C-9), 158.4 (C-3), 131.9 (C-5), 125.1 (C-2), 123.7 (C-4), 96.0 (C-7), 73.7 (C-6), 29.8 (C-10), 4.2 (C-8), 103.5 (C-1′), 78.5 (C-5′), 78.1 (C-3′), 74.8 (C-2′), 71.1 (C-4′), 62.3 (C-6′); HR-ESI-MS *m/z* 365.0665 $[M + Na]^+$ (calcd for $C_{15}H_{18}O_7SNa$, 365.0665).

3.4 Acid hydrolysis and gas chromatographic analysis of 3

Compound **3** (2 mg) was hydrolyzed with 2 M HCl for 4 h at 80°C. The reaction mixture was evaporated under vacuum to yield a residue, which was then dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer was concentrated and dried by N_2, and treated with dry pyridine (1 ml) and L-cysteine methyl ester hydrochloride (2 mg), followed by heating at 60°C for 2 h, and then concentrated to dryness with N_2. The residue was added to *N*-(trimethylsilyl)imidazole (0.2 ml) and kept at 60°C for 1 h. Then, the solution was diluted with H_2O (1 ml) and extracted with cyclohexane (3 × 1 ml). The supernatant was concentrated to 1 ml, then subjected to GC analysis [column: AT-SE-30 (0.5 μm × 0.32 mm × 30 m), detector: FID, column temperature: 220°C, detector temperature: 270°C, injector temperature: 270°C, and carried gas: N_2]. A peak of the derivative of **3** was observed at t_R 28.78 min (D-Glc), whereas the peaks of the standard monosaccharide derivatives were recorded at 28.76 (D-Glc) and 30.22 (L-Glc), respectively.

Acknowledgements

This work was financially supported by grants from the Program for Changjiang Scholars and Innovative Research Team in University (IRT0965), the Natural Science Foundation of Guangdong Province (No. 8351063201000003; No. 9451052005001975), the Science and Technology Development Fund of Macau (No. 013/2008/A1), and Administration of Traditional Chinese Medicine of Guangdong Province (No. 2010235).

Note

1. The authors contributed equally to this work.

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