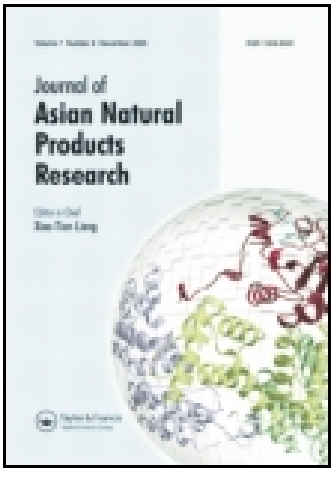

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Aromatic acid derivatives from the lateral roots of Aconitum carmichaelii

Zhi-Bo Jiang[a], Bing-Ya Jiang[a], Cheng-Gen Zhu[a], Qing-Lan Guo[a], Ying Peng[a], Xiao-Liang Wang[a], Sheng Lin[a] & Jian-Gong Shi[a]
[a] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China
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Aromatic acid derivatives from the lateral roots of *Aconitum carmichaelii*

Zhi-Bo Jiang, Bing-Ya Jiang, Cheng-Gen Zhu, Qing-Lan Guo, Ying Peng, Xiao-Liang Wang, Sheng Lin and Jian-Gong Shi*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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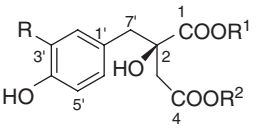
Seven new aromatic acid derivatives (**1**–**7**), together with five known analogs, were isolated from the lateral roots of *Aconitum carmichaelii*. Structures of the new compounds were determined by spectroscopic and chemical methods as 4-methyl (−)-(*R*)-hydroxyeucomate (**1**), 4-butyl (−)-(*R*)-hydroxyeucomate (**2**), 4-butyl-1-methyl (+)-(*R*)-2-*O*-(4′-hydroxy-3′-methoxybenzoyl)malate (**3**), 1-butyl-4-methyl (+)-(*R*)-2-*O*-(4′-hydroxy-3′-methoxybenzoyl)malate (**4**), dimethyl (+)-(*R*)-2-*O*-(4′-hydroxy-3′-methoxybenzoyl)malate (**5**), dimethyl (+)-(*R*)-2-*O*-(4′-hydroxybenzoyl)malate (**6**), and methyl (±)-3-(4′-hydroxy-3′-methoxyphenyl)-3-sulfopropionate (**7**), respectively. Compounds **1** and **2** are 2-benzylmalates (eucomate derivatives), **3**–**6** belong to 2-*O*-benzoylmalates, and **7** is a rare phenylpropionate containing a sulfonic acid group. The absolute configurations of eucomate derivatives were confirmed by X-ray crystallographic analysis of 4-methyl eucomate (**11**).

Keywords: *Aconitum carmichaelii*; Ranunculaceae; (*R*)-2-benzylmalates; (*R*)-2-*O*-benzoylmalates; methyl 3-(4′-hydroxy-3′-methoxyphenyl)-3-sulfopropionate

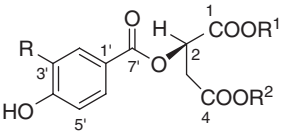
1. Introduction

The lateral roots of *Aconitum carmichaelii* Debx. (Ranunculaceae), known as 'fu zi', are one of the most important herbal drugs in traditional Chinese medicine [1]. They are used in raw or prepared forms to treat various diseases such as cadianeuria, neuralgia, and rheumatalgia in China, Japan, and Korea [2]. Previous studies have shown that toxic aconitine C_{19}-diterpenoid alkaloids are the main active constituents of these drugs, and more than 40 aconitine alkaloids have been isolated from the raw and prepared forms of 'fu zi' [3]. However, the nonalkaloid constituents have rarely been considered. As part of a program to systematically study the chemical diversity of traditional Chinese medicines and their biological effects [4], a water extract of the raw lateral roots of *A. carmichaelii* was investigated. In previous work, 4 new C_{20}- and 22 new C_{19}-diterpenoid alkaloids, together with two known analogs, were characterized in several fractions obtained from the extract [5,6]. Herein, the remaining fractions of the same extract were investigated, leading to the isolation of seven new aromatic acid derivatives (**1**–**7**), along with five previously known analogs (Figure 1). Compounds **1** and **2** are (*R*)-2-benzylmalates, **3**–**6** may be classified as (*R*)-2-*O*-benzoylmalate derivatives, and **7** is a rare phenylpropionate containing a sulfonic acid group. In this paper, we describe the isolation and structure elucidation of the new compounds.

*Corresponding author. Email: shijg@imm.ac.cn

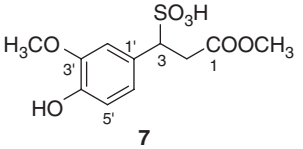


1 R = OH, R^1 = H, R^2 = CH_3
2 R = OH, R^1 = H, R^2 = n-But
8 R = OH, R^1 = R^2 = H
9 R = R^1 = R^2 = H
10 R = H, R^1 = R^2 = CH_3
11 R = R^1 = H, R^2 = CH_3



3 R = OCH_3, R^1 = CH_3, R^2 = n-But
4 R = OCH_3, R^1 = n-But, R^2 = CH_3
5 R = OCH_3, R^1 = R^2 = CH_3
6 R = H, R^1 = R^2 = CH_3

1" 2" 3" 4"
n-But = $CH_2CH_2CH_2CH_3$



7

Figure 1. The structures of compounds **1**–**11**.

2. Results and discussion

Compound **1** was obtained as a colorless gum with $[\alpha]_D^{20}$ −42.5 (*c* 0.18, MeOH). The IR spectrum of **1** showed absorption bands for hydroxyl (3390 cm^{-1}), carbonyl (1725 cm^{-1}), and aromatic (1608 and 1526 cm^{-1}) functional groups. The molecular formula $C_{12}H_{14}O_7$ was indicated by HR-ESI-MS at *m/z* 269.0658 $[M - H]^-$, combined with the NMR spectral data. The ^1H NMR spectrum of **1** in acetone-d_6 showed signals attributed to a *meta*–*para*-trisubstituted phenyl group at δ_H 6.79 (d, J = 1.5 Hz, H-2′), 6.69 (d, J = 8.5 Hz, H-5′), and 6.58 (dd, J = 8.5, 1.5 Hz, H-6′); two methylenes at δ_H 2.95 (d, J = 15.5 Hz, H-3a), 2.62 (d, J = 15.5 Hz, H-3b), 2.90 (d, J = 14.0 Hz, H-7′a), and 2.82 (d, J = 14.0 Hz, H-7′b); and a methoxy group at δ_H 3.58 (s). The ^{13}C NMR and DEPT spectra of **1** showed carbon signals (Table 1) corresponding to the above units including two oxygen-bearing aromatic carbons [δ_C 145.2 (C-3′) and 144.8 (C-4′)], in addition to two carbonyl carbons [δ_C 175.8 (C-1) and 171.4 (C-4)] and an oxygen-bearing sp^3-hybridized quaternary carbon [δ_C 76.1 (C-2)]. These spectroscopic data suggest that **1** is a methyl monoester of hydroxyeucomic acid (**8**) [7]. The suggestion was supported by comparing the NMR data of **1** with those of the co-occurring (−)-(*R*)-eucomic acid analogs (**9**–**11**) and was further confirmed by 2D NMR data analysis. The HMQC and ^1H–^1H COSY spectra of **1** provided unambiguous assignments of proton and carbon signals in the NMR spectra. In the HMBC spectrum, correlations from H_2-3 to C-1, C-2, and C-4, combined with the chemical shifts of these proton and carbon resonances, revealed the presence of a 2-substituted malic acid moiety in **1**. HMBC correlations of H-2′/C-4′, C-6′, and C-7′; H-5′/C-3′ and C-4′; H-6′/C-2′, C-4′, and C-7′; and H_2-7′/C-1′, C-2′, and C-6′, together with their chemical shifts, verified the occurrence of a 3′,4′-dihydroxybenzyl moiety. In addition, HMBC correlations from H_2-3 to C-7′ and from H_2-7′ to C-1, C-2, and C-3 confirmed that the 3′,4′-dihydroxybenzyl moiety was located at C-2 of the malic acid moiety. The HMBC correlation from OCH_3 to C-4 demonstrated that the malic acid was esterified at the 4-carboxyl moiety. Accordingly, the planar structure of compound **1** was determined as 4-methyl hydroxyeucomate.

Table 1. ^{1}H and ^{13}C NMR spectral data (δ) for compounds **1**–**7**.

	1		**2**[a]		**3**[a]		**4**[a]		**5**		**6**[a]		**7**[a]	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		175.8		178.0		170.4		169.8		169.9		170.3		173.3
2a		76.1		77.2	5.59 dd (7.5, 5.5)	69.6	5.60 dd (7.5, 5.5)	69.6	5.69 t (6.0)	68.6	5.60 dd (7, 5)	69.4	3.32 m	38.1
2b													3.01 dd (15.6, 11.2)	
3a	2.95 d (15.5)	43.2	2.86 d (15.5)	44.3	3.05 dd (15.5, 5.5)	36.6	3.05 d (15.5, 5.5)	36.9	3.04 d (16.0, 6.0)	36.1	3.03 d (5.0)	36.6	4.33 m	62.8
3b	2.62 d (15.5)		2.49 d (15.5)		3.01 dd (15.5, 7.5)		3.01 d (15.5, 7.5)		3.01 d (16.0, 6.0)		3.01 d (7.0)			
4		171.4		172.6		169.6		170.1		169.6		170.1		
1′		128.0		128.6		121.6		121.6		121.0		121.3		128.9
2′	6.79 d (1.5)	118.5	6.61 d (1.5)	119.1	7.54 d (1.5)	113.4	7.54 d (1.5)	113.4	7.54 brs	112.0	7.89 d (8.5)	132.8	7.03 d (1.2)	114.0
3′		145.2		146.1		148.1		148.1		146.2	6.92 d (8.5)	116.1		148.6
4′		144.8		145.6		152.5		152.6		150.6		163.5		147.3
5′	6.69 d (8.5)	115.5	6.56 d (7.5)	116.2	6.92 d (8.5)	115.6	6.92 d (8.5)	115.6	6.93 d (8.0)	114.1	6.92 d (8.5)	116.1	6.72 d (7.8)	115.7
6′	6.58 dd (8.5, 1.5)	122.8	6.46 dd (7.5, 1.5)	123.3	7.58 dd (8.5, 1.5)	125.0	7.58 dd (8.5, 1.5)	125.0	7.64 dd (8.0, 1.5)	124.7	7.89 d (8.5)	132.8	6.85 dd (7.8, 1.2)	123.2
7′a	2.90 d (14.0)	45.2	2.80 d (14.0)	46.1		165.7		165.6		165.2		165.6		
7′b	2.82 d (14.0)		2.70 d (14.0)											
OCH_3-1					3.68 s	52.2			3.72 s	52.1	3.68 s	52.2	3.56 s	52.3
OCH_3-4	3.58 s	51.7					3.74 s	52.7	3.76 s	52.7	3.73 s	52.7		
OCH_3-3′					3.89 s	56.3	3.89 s	56.3	3.92 s	56.1			3.85 s	56.4

Notes: NMR data (δ) were measured in acetone-d_6 for **1**, **3**, **4**, and **6** in MeOH-d_4 for **2** and **7**, and in $CDCl_3$ for **5**, at 500 MHz for ^{1}H and 125 MHz for ^{13}C (**1**–**6**) and at 600 MHz for ^{1}H and 150 MHz for ^{13}C (**7**). Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H–^{1}H COSY, HSQC, and HMBC experiments.

[a] Data for the butoxy moiety in **2**–**4**. **2**: δ_H 3.96 (2H, t, J = 6.5 Hz, H-1″), 1.49 (2H, m, H-2″), 1.28 (2H, m, H-3″), 0.83 (3H, t, J = 7.5 Hz, H-4″); δ_C 65.8 (C-1″), 32.0 (C-2″), 20.4 (C-3″), 14.3 (C-4″). **3**: δ_H 4.16 (2H, t, J = 6.5 Hz, H-1″), 1.61 (2H, m, H-2″), 1.37 (2H, m, H-3″), 0.89 (3H, t, J = 7.5 Hz, H-4″); δ_C 65.8 (C-1″), 31.2 (C-2″), 19.6 (C-3″), 13.8 (C-4″). **4**: δ_H 4.11 (2H, t, J = 6.5 Hz, H-1″), 1.58 (2H, m, H-2″), 1.35 (2H, m, H-3″), 0.87 (3H, t, J = 7.5 Hz, H-4″); δ_C 65.2 (C-1″), 31.4 (C-2″), 19.7 (C-3″), 13.9 (C-4″).

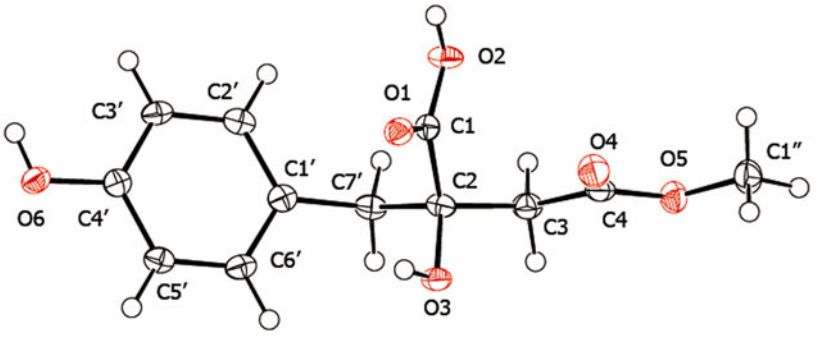


Figure 2. ORTEP diagram of compound **11**.

The similarities of the negative optical rotations for **1** with those of hydroxyeucomic acid (**8**) [7], (−)-(*R*)-eucomic acid (**9**) [8], dimethyl eucomate (**10**) [7,8], and yunnanensin C [9] suggest that **1** possesses the same absolute configuration as these known natural products. Because only the absolute configuration of **9** among the known analogs had been determined by synthetic correlation with piscidic acid [8], an available single crystal of the co-occurring 4-methyl eucomate (**11**) {$[\alpha]_D^{20}$ − 18.9 (*c* 0.23, MeOH)}, which had been previously reported as methyl eucomate without optical rotation data [10], was analyzed by X-ray crystallography using Cu K_α radiation. An ORTEP drawing, with the atom numbering indicated, is shown in Figure 2. The absolute configuration was determined on the basis of the Flack parameter of −0.1 (3), which supported the configuration assignment for **1**. Therefore, the structure of compound **1** was determined as 4-methyl (−)-(*R*)-hydroxyeucomate.

Compound **2** was obtained as a colorless gum with $[\alpha]_D^{20}$ − 21.8 (*c* 0.23, MeOH). The molecular formula $C_{15}H_{20}O_7$ was indicated from HR-ESI-MS at *m/z* 313.1280 $[M + Na]^+$, combined with the NMR spectral data. The UV, IR, and NMR spectroscopic features of **2** (see Section 3 and Table 1) were similar to those of **1**. Comparison of the NMR spectral data of **2** and **1** indicated that the methoxy group in **1** was replaced by a butoxy group in **2**. This demonstrated that **2** was the butyl monoester of hydroxyeucomic acid, which was confirmed by 2D NMR data analysis and hydrolysis. In particular, the $^1H-^1H$ COSY correlations of H_2-1″/H_2-2″/H_2-3″/H_3-4″ and the HMBC correlation of H_2-1″/C-4 verified that the butoxy group was also incorporated in the 4-carboxylic acid in **2**. Hydrolysis of **2** with 0.2 N NaOH produced hydroxyeucomic acid (**8**), which exhibited ^1H NMR, HR-ESI-MS, and $[\alpha]_D^{20}$ data consistent with those in the literature [7]. Therefore, the structure of compound **2** was determined as 4-butyl (−)-(*R*)-hydroxyeucomate.

Compound **3**, a colorless gum with $[\alpha]_D^{20}$ + 12.8 (*c* 0.27, MeOH), has the molecular formula $C_{17}H_{22}O_8$ as indicated by HR-ESI-MS at *m/z* 355.1390 $[M + H]^+$ and the NMR spectral data. The NMR spectral data (Table 1) showed the presence of a disubstituted benzoyl, a butoxy, and two methoxy groups, as well as a malic acid moiety in **3**. This was confirmed by 2D NMR data analysis. The proton and carbon signals in the NMR spectra were unambiguously assigned by the HMQC and $^1H-^1H$ COSY experiments. The vicinal coupling COSY correlation of H-2/H_2-3 and two- and three-bond HMBC correlations from H-2 to C-1, C-3, and C-4 and from H_2-3 to C-1, C-2, and C-4, in combination with the chemical shifts of these proton and carbon resonances, confirmed the malic acid moiety in **3**. The

COSY correlation of H-5′/H-6′ and HMBC correlations of H-2′/C-1′, C-3′, C-4′, C-6′, and C-7′; H-5′/C-1′, C-3′, and C-4′; and H-6′/C-2′, C-4′, and C-7′, together with their chemical shifts, verified the 3′,4′-disubstituted benzoyl unit. The butoxy group was confirmed by the COSY correlations of H_2-1″/H_2-2″/H_2-3″/H_3-4″ and HMBC correlations of H_2-1″/C-2″ and C-3″; H_2-2″/C-1″, C-3″, and C-4″; H_2-3″/C-1″, C-2″, and C-4″; and H_3-4″/C-2″ and C-3″. In addition, the HMBC correlations from H-2 to C-7′ and from H_2-1″ to C-4 revealed that the benzoyl and butyl units were incorporated as esters at the 2-OH and 4-carboxyl groups of the malic acid moiety in **3**, respectively. The HMBC correlations of 3′-OCH_3/C-3′ and 1-OCH_3/C-1 demonstrated that the two methoxy groups were located at C-3′ and C-1 in **3**. The absolute configuration of compound **3** was determined by comparing its specific rotation with the reported data for the known compounds, (*S*)-benzoyl malic acid, and (*R*)-2-*O*-galloyl malic acid dimethyl ester [11]. The specific rotation of (*S*)-benzoyl malic acid has a negative value $[\alpha]_D^{21}$ − 7 (*c* 1.0, $CHCl_3$), whereas that of (*R*)-galloyl malic acid dimethyl ester is positive $[\alpha]_D^{20}$ + 21 (*c* 0.1, MeOH) [11]. Therefore, compound **3** was assigned the *R* configuration based on its positive specific rotation, and its structure was designated as 4-butyl-1-methyl (+)-(*R*)-2-*O*-(4′-hydroxy-3′-methoxybenzoyl)malate.

Compound **4**, a colorless gum with $[\alpha]_D^{20}$ + 17.0 (*c* 0.41, MeOH), is an isomer of **3** as indicated from the spectroscopic data. Comparison of the NMR data for **4** with those of **3** indicated that the proton and carbon resonances of the ester methoxy group in **4** were deshielded by $\Delta\delta_H$ + 0.06 ppm and $\Delta\delta_C$ + 0.5 ppm, respectively, whereas the oxymethylene resonances of the butoxy group in **4** were shielded by $\Delta\delta_H$ −0.05 ppm and $\Delta\delta_C$ −0.6 ppm, respectively. This suggested that the 4-butyl-1-methyl ester in **3** was replaced by a 1-butyl-4-methyl ester in **4**. This was confirmed by the HMBC correlations of 4-OCH_3/C-4 and H_2-1″ to C-1 in the HMBC spectrum of **4**. Compound **4** displayed a specific rotation similar to that of **3**, indicating that **4** had the same *R* configuration. Therefore, the structure of compound **4** was determined as 1-butyl-4-methyl (+)-(*R*)-2-*O*-(4′-hydroxy-3′-methoxybenzoyl)malate.

Compound **5**, a colorless gum with $[\alpha]_D^{20}$ + 31.4 (*c* 0.70, MeOH), has the molecular formula $C_{14}H_{16}O_8$ as indicated by HR-ESI-MS at *m/z* 313.0916 $[M + H]^+$ and the NMR spectral data. Comparison of the NMR spectral data of **5** with those of **4** (Table 1) indicated replacement of the butoxy group in **4** by a methoxy group in **5**, which was confirmed by 2D NMR data analysis of **5**. In particular, the HMBC correlations of 1-OCH_3/C-1 and 4-OCH_3/C-4 verified that both the 1- and 4-carboxyl groups of the malic acid moiety in **5** were methyl ester. The *R* configuration was indicated by its specific rotation similar to those of **3** and **4**. Therefore, the structure of compound **5** was determined as dimethyl (+)-(*R*)-2-*O*-(4′-hydroxy-3′-methoxybenzoyl)malate.

Compound **6** was obtained a colorless gum with $[\alpha]_D^{20}$ + 5.5 (*c* 0.11, MeOH). Its molecular formula $C_{13}H_{14}O_7$ was indicated by HR-ESI-MS at *m/z* 281.0661 $[M - H]^-$ and the NMR spectral data. Comparison of the NMR spectral data between **6** and **5** (Table 1) showed replacement of the 4′-hydroxy-3′-methoxybenzoyl unit in **5** by a 4′-hydroxybenzoyl unit in **6**. This was further confirmed by 2D NMR data analysis of **6**. Particularly, the HMBC correlations of C-7′ with H-2, H-2′, and H-6′; 1-OCH_3/C-1; and 4-OCH_3/C-4, together with their shifts, confirmed that the 4′-hydroxybenzoyl unit and the two methoxy groups were incorporated as esters at the 2-OH and the 1- and 4-carboxyl groups of the malic acid moiety in **6**, respectively. The positive optical rotation of **6**, similar to those of **3**–**5**, indicated the *R* configuration. Therefore,

compound **6** was determined as dimethyl (+)-(*R*)-2-*O*-(4′-hydroxybenzoyl)malate.

Compound **7** was obtained a white amorphous powder with $[\alpha]_D^{20}$ 0 (*c* 0.26, MeOH). Its molecular formula $C_{11}H_{14}O_7S$ was indicated by HR-ESI-MS at *m/z* 313.0353 $[M + Na]^+$ and the NMR spectral data. NMR data analysis (Table 1) revealed the presence of a methine, a methylene, a carbonyl, and two methoxy groups, in addition to a 4′-hydroxy-3′-methoxyphenyl moiety. This, combined with the molecular composition, suggested that **7** was an unusual methyl (4′-hydroxy-3′-methoxyphenyl)propionate derivative containing a sulfo group, which was further confirmed by 2D NMR data. The signals in the NMR spectra were assigned by the HMQC and $^1H-^1H$ COSY experiments of **7**. The COSY correlations of H_2-2/H-3 and the HMBC correlations from H_2-2 to C-1 and C-3 and from 1-OCH_3 to C-1 verified the presence of a substituted methyl propionate moiety. The COSY correlations of H-5′/H-6′ and the HMBC correlations of H-2′/C-1′, C-3′, C-4′, and C-6′; H-5′/C-1′, C-3′, C-4′, and C-6′; H-6′/C-2′, C-4′, and C-5′; and from 3′-OCH_3/C-3′ verified the presence of the 4′-hydroxy-3′-methoxyphenyl moiety. In addition, the HMBC correlations from H_2-2 to C-1′ and from H-2′ and H-6′ to C-3 demonstrated that both the 4′-hydroxy-3′-methoxyphenyl moiety and the sulfo group were located at C-3 of the methyl propionate moiety in **7**. This was supported by comparing the NMR data of **7** with those of synthetic analogs reported in the literature [12]. Compound **7** is optically inactive, indicating that it was obtained as a racemate. Therefore, the structure of compound **7** was determined as methyl (±)-3-(4′-hydroxy-3′-methoxyphenyl)-3-sulfopropionate.

The known compounds were identified by comparison of their spectroscopic data with those reported in the literature as (−)-(*R*)-hydroxyeucomic acid (**8**) [7], (−)-(*R*)-eucomic acid (**9**) [8], dimethyl (−)-(*R*)-eucomate (**10**) [8], 4-methyl eucomate (**11**) [10], ferulic acid [13], methyl ferulate [14], butyl ferulate [15], methyl 4-*O*-β-D-glucopyranosylferulate [16], and linocinnamarin [17].

The methyl and butyl esters, including **1–7**, were considered natural products because methylation or butylation of the acids **8** and **9** was unlikely to occur during the isolation procedure. Furthermore, esterification did not proceed under simulated conditions heating MeOH or *n*-BuOH solutions of the acids or H_2O solutions of the esters, either with or without silica gel, MCI gel, Sephadex LH-20, or reversed C_{18} silica gel at 45°C for 48 h. Although the HPLC–ESI-MS technique was used to detect the occurrence of these compounds in the crude extract, none were observed, possibly due to their low contents and the limit of the ESI-MS detection technique.

All the compounds isolated in this study were assayed for their neuroprotective effects against SK-N-SH neuroblastoma cells [18]. As compared with the blank control, at a concentration of 10 μM, (−)-(*R*)-eucomate (**10**) and butyl ferulate showed protective activity against oxygen-glucose deprivation-induced neurotoxicity, with cell survival rates improved by 39.96% ± 4.63% and 23.39% ± 3.48%, respectively. Methyl ferulate and linocinnamarin exhibited protective activity against L-glutamic acid-induced neurotoxicity, with cell survival rates improved by 14.03% ± 4.50% and 11.17% ± 3.48%, respectively. The isolates were also assessed for their activities against DL-galactosamine-induced WB-F344 cell damage [19], and nitric oxide production in mouse peritoneal macrophages [20], as well as for their cytotoxicity against several human cancer cell lines [21], but were found to be inactive at 10 μM.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on P-2000 polarimeter (JASCO, Tokyo, Japan). UV spectra were measured on a JASCOP-650 spectrometer (JASCO). IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission) (Thermo Electron Corporation, Madison, WI, USA). NMR spectra were obtained at 500 or 600 MHz for ^{1}H, and 125 or 150 MHz for ^{13}C on Inova 500 or SYS 600 spectrometers (Varian Associates Inc., Palo Alto, CA, USA) in acetone-d_6, MeOH-d_4, or $CDCl_3$ with solvent peaks used as references. ESI-MS and HR-ESI-MS data were measured using an AccuToFCS JMS-T100CS spectrometer (Agilent Technologies, Ltd, Santa Clara, CA, USA). Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector (Waters Corporation, Milford, MA, USA), with a YMC-Pack Ph (250 mm × 10 mm i.d.) column packed with phenyl-silica gels (5 μm) (YMC Co., Ltd, Kyoto, Japan). TLC was carried out with glass precoated silica gel GF_{254} plates (Qingdao Marine Chemical Inc.). Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating. Unless otherwise noted, all chemicals were obtained from commercially available sources and were used without further purification.

3.2 Plant material

The lateral root of *A. carmichaelii* Debx was collected in June 2009 from the culture field in Jiangyou, Sichuan Province, China. Plant identity was verified by Dr Yan Ren (Chengdu University of Traditional Chinese Medicine, Sichuan 610075, China). A voucher specimen (no. ID-S-2383) was deposited at the herbarium of the Department of Chemistry of Natural Products, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China.

3.3 Extraction and isolation

The air-dried lateral roots of *A. carmichaelii* (50 kg) were powdered and extracted with H_2O (3 × 150 L × 6 h) at 40°C. The H_2O extract was concentrated to 120 L under reduced pressure, subjected to chromatography over a macroporous adsorbent resin (HPD-110, 19 kg) column (20 cm × 200 cm), and eluted successively with H_2O (50 L), 30% EtOH (120 L), 50% EtOH (120 L), and 95% EtOH (100 L) to afford the corresponding fractions A–D. After removal of the solvent, fraction C (3.5 kg) was chromatographed over MCI gel CHP 20P with successive elution using H_2O (10 L), 30% EtOH (30 L), 50% EtOH (20 L), and 95% EtOH (10 L) to give fractions C1–C4. Fraction C2 (600 g) was again fractionated via MCI gel CHP 20P with successive elution using H_2O (10 L), 30% EtOH (30 L), 50% EtOH (20 L), and 95% EtOH (10 L) to give subfractions C2-1–C2-4. Subfraction C2-1 (200 g) was dissolved in H_2O (500 mL), basified with concentrated ammonium hydroxide (25 mL) to pH 10, and then extracted with EtOAc (500 mL × 4). The aqueous layer was acidified with 6.0 N HCl (66 mL) to pH 4, and partitioned with *n*-butanol (500 mL × 3). The *n*-butanol phase was concentrated under reduced pressure to give a residue, C-2-B (12 g), which was chromatographed over silica gel (100 g) eluting with a gradient of $CHCl_3$–MeOH (95:5–85:15) to give fractions C-2-B-1–C-2-B-6. Fraction C-2-B-3 (3.9 g) was separated by reversed-phase medium pressure liquid chromatography (RP-MPLC, 30% MeCN

in H_2O, containing 0.1% TFA) to give C-2-B-3-1–C-2-B-3-6, of which C-2-B-3-3 (500 mg) was further separated by RP HPLC (40% MeCN in H_2O, containing 0.1% TFA, 1.5 mL/min) to yield **1** (10.5 mg, $t_R = 18.3$ min) and **2** (4.1 mg, $t_R = 22.2$ min). Fraction C-4-B-3-4 (300 mg) was chromatographed over silica gel (petroleum ether–EtOAc, 50:1–10:1), followed by semi-preparative RP HPLC purification (70% MeOH in H_2O, 1.5 mL/min) to give **3** (7.0 mg, $t_R = 41.3$ min), **4** (5.2 mg, $t_R = 43.7$ min), methyl ferulate (65.0 mg, $t_R = 17.1$ min), and butyl ferulate (73.5 mg, $t_R = 20.0$ min). Fraction C-2-B-3-5 (200 mg) was successively chromatographed over Sephadex LH-20 (petroleum ether–$CHCl_3$–MeOH, 5:5:1) and silica gel (petroleum ether–EtOAc, 15:1), followed by RP-HPLC separation (75% MeOH in H_2O, 1.5 mL/min) to give **5** (24.2 mg, $t_R = 26.5$ min), **6** (15.6 mg, $t_R = 32.0$ min), **10** (8.5 mg, $t_R = 36.3$ min), and **11** (63.0 mg, $t_R = 17.0$ min). Fraction C-2-B-6 (5.3 g) was chromatographed over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to afford C-2-B-6-1–C-2-B-6-3. Fraction C-2-B-6-1 (600 mg) was further chromatographed over Sephadex LH-20 eluting with petroleum ether–$CHCl_3$–MeOH (5:5:1), and then purified by RP-HPLC (15% MeCN in H_2O, containing 0.1% TFA, 1.5 mL/min) to yield methyl 4-*O*-β-D-glucopyranosylferulate (40.0 mg, $t_R = 23.2$ min) and linocinnamarin (60.2 mg, $t_R = 37.1$ min). Fraction C-2-B-6-3 (330 mg) was isolated by preparative TLC (mobile phase: $CHCl_3$–MeOH, 4.5:1), followed by RP-HPLC separation (10% MeCN in H_2O, 1.5 mL/min) to yield **7** (3.7 mg, $t_R = 21.0$ min), **8** (6.7 mg, $t_R = 30.0$ min), **9** (8.2 mg, $t_R = 35.5$ min), and ferulic acid (20.3 mg, $t_R = 24.8$ min).

*3.3.1 4-Methyl (−)-(R)-hydroxyeucomate (**1**)*

Colorless gum; $[\alpha]_D^{20}$ − 42.5 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.44), 232 (sh, 4.10), 282 (3.93) nm; IR ν_{max}: 3390, 2968, 1725, 1676, 1608, 1527, 1443, 1362, 1286, 1202, 1149, 1121, 1046, 1000, 963, 877,796 cm^{-1}; for ^1H NMR (acetone-d_6, 500 MHz) spectral data, see Table 1; for ^{13}C NMR (acetone-d_6, 125 MHz) spectral data, see Table 1; ESI-MS: *m/z* 269 $[M - H]^-$; HR-ESI-MS: *m/z* 269.0658 $[M - H]^-$ (calcd for $C_{12}H_{13}O_7$, 269.0667).

*3.3.2 4-Butyl (−)-(R)-hydroxyeucomate (**2**)*

Colorless gum; $[\alpha]_D^{20}$ − 21.8 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.22), 227 (3.60), 282 (3.25) nm; IR ν_{max}: 3464, 3325, 3224, 2961, 1747, 1716, 1617, 1598, 1515, 1440, 1376, 1277, 1218, 1178, 1114, 1046, 990, 839, 780 cm^{-1}; for ^1H NMR (MeOH-d_4, 500 MHz) spectral data, see Table 1; for ^{13}C NMR (MeOH-d_4, 125 MHz) spectral data, see Table 1; ESI-MS: *m/z* 335 $[M + Na]^+$; HR-ESI-MS: *m/z* 313.1280 $[M + H]^+$ (calcd for $C_{15}H_{21}O_7$, 313.1282), 335.1104 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_7Na$, 335.1101).

*3.3.3 4-Butyl-1-methyl (+)-(R)-2-O-(4′-hydroxy-3′-methoxybenzoyl)malate (**3**)*

Colorless gum; $[\alpha]_D^{20}$ + 12.8 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε): 204 (3.62), 220 (3.55), 265 (3.29), 295 (3.07) nm; IR ν_{max}: 3366, 2919, 2851, 1686, 1598, 1516, 1457, 1390, 1288, 1204, 1141, 1056, 1030, 838, 801,723 cm^{-1}; for ^1H NMR (acetone-d_6, 500 MHz) spectral data, see Table 1; for ^{13}C NMR (acetone-d_6, 125 MHz) spectral data, see Table 1; ESI-MS: *m/z* 377 $[M + Na]^+$; HR-ESI-MS: *m/z* 355.1390 $[M + H]^+$ (calcd for $C_{17}H_{23}O_8$, 355.1387), 377.1210 $[M + Na]^+$ (calcd for $C_{17}H_{22}O_8Na$, 377.1207).

*3.3.4 1-Butyl-4-methyl (+)-(R)-2-O-(4′-hydroxy-3′-methoxybenzoyl)malate (**4**)*

Colorless gum; $[\alpha]_D^{20}$ + 17.0 (*c* 0.41, MeOH); UV (MeOH) λ_{max} (log ε): 204

(4.10), 218 (4.07), 265 (3.87), 295 (sh, 3.70) nm; IR ν_{max}: 3350, 2957, 2919, 2850, 1734, 1681, 1635, 1464, 1207, 1181, 1138, 837, 801, 722 cm^{-1}; for ^1H NMR (acetone-d_6, 500 MHz) spectral data, see Table 1; for ^{13}C NMR (acetone-d_6, 125 MHz) spectral data, see Table 1; ESI-MS: *m/z* 377 $[M + Na]^+$; HR-ESI-MS: *m/z* 355.1391 $[M + H]^+$ (calcd for $C_{17}H_{23}O_8$, 355.1387), 377.1210 $[M + Na]^+$ (calcd for $C_{17}H_{22}O_8Na$, 377.1207).

3.3.5 *Dimethyl (+)-(R)-2-O-(4′-hydroxy-3′-methoxybenzoyl)malate (**5**)*

Colorless gum; $[\alpha]_D^{20} + 31.4$ (*c* 0.70, MeOH); UV (MeOH) λ_{max} (log ε): 197 (4.51), 265 (3.95) nm; IR ν_{max}: 3420, 3009, 2956, 2919, 2850, 1740, 1720, 1597,1515, 1434, 1375, 1284, 1211, 1110, 1055, 1029, 878, 782, 762 cm^{-1}; for ^1H NMR ($CDCl_3$, 500 MHz) spectral data, see Table 1; for ^{13}C NMR ($CDCl_3$, 125 MHz) spectral data, see Table 1; ESI-MS: *m/z* 335.1 $[M + Na]^+$; HR-ESI-MS: *m/z* 313.0916 $[M + H]^+$ (calcd for $C_{14}H_{17}O_8$, 313.0918), 335.0738 $[M + Na]^+$ (calcd for $C_{14}H_{16}O_8Na$, 335.0737).

3.3.6 *Dimethyl (+)-(R)-2-O-(4′-hydroxy-3′-methoxybenzoyl)malate (**6**)*

Colorless gum; $[\alpha]_D^{20} + 5.5$ (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε): 202 (3.62), 217 (3.21), 259 (3.10) nm; IR ν_{max}: 3393, 3213, 2919, 2850, 1730, 1679, 1647, 1608, 1516, 1468, 1443, 1420, 1204, 1140, 1050, 802, 724 cm^{-1}; for ^1H NMR (acetone-d_6, 500 MHz) spectral data, see Table 1; for ^{13}C NMR (acetone-d_6, 125 MHz) spectral data, see Table 1; ESI-MS: *m/z* 305 $[M + Na]^+$; HR-ESI-MS: *m/z* 281.0661 $[M - H]^-$ (calcd for $C_{13}H_{13}O_7$, 281.0667).

3.3.7 *Methyl (±)-3-(4′-hydroxy-3′-methoxyphenyl)-3-sulfopropionate (**7**)*

White amorphous powder; $[\alpha]_D^{20}$ 0 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.23), 231 (3.67), 281 (3.20) nm; IR ν_{max}: 3222, 2920, 2851, 1709, 1679, 1605, 1526, 1438, 1373, 1250, 1203, 119, 1045, 802, 724, 646 cm^{-1}; for ^1H NMR (MeOH-d_4, 600 MHz) spectral data, see Table 1; for ^{13}C NMR (MeOH-d_4, 150 MHz) spectral data, see Table 1; ESI-MS: *m/z* 289 $[M - H]^-$;HR-ESI-MS: *m/z* 308.0799 $[M + NH_4]^+$ (calcd for $C_{11}H_{18}O_7NS$, 308.0798), 313.0353 $[M + Na]^+$ (calcd for $C_{11}H_{14}O_7SNa$, 313.0352).

3.3.8 *X-ray crystallography of **11***

$C_{12}H_{14}O_6$, $M = 254.23$, orthorhombic, $P2_12_12_1$, $a = 5.930$ (3) Å, $b = 6.793$ (2) Å, $c = 29.104$ (5) Å, $\alpha = \beta = \gamma = 90^o$, $V = 1172.4$ (7) $Å^3$, $Z = 4$, $D_{calcd} = 1.44\ mg\ mm^{-3}$, 3660 collected reflections, 2102 reflections independent, $R_1 = 0.0427$, $wR_2 = 0.1045$. The data were collected on an Agilent Xcalibur Eos Gemini diffractometer with Cu K_α radiation ($\mu = 0.994$) by using the ω scan technique with 2θ from 12.16° to 138.64°. The crystal structures were solved by direct methods by using SHELXS-97, and all nonhydrogen atoms were refined anisotropically using the least-squares method. All hydrogen atoms were positioned by geometrical calculations and difference Fourier overlapping calculation. The absolute configuration was determined on the basis of the Flack parameter −0.1(3). Crystallographic data for the structure of **11** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication (CCDC 988618). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

3.3.9 *Hydrolysis of **2***

A solution of **2** (7.3 mg) was hydrolyzed with 0.2 N NaOH (5.0 mL) at 40°C for 0.5 h. The reaction mixture was acidified

with 0.5 N HCl to pH 2, and evaporated to dryness under reduced pressure. The residue was chromatographed on a Sephadex LH-20 column with elution by MeOH–H_2O (1:1), followed by RP-HPLC purification using the mobile phase MeOH–H_2O (7:93) containing 0.1% TFA to yield **8**: $[\alpha]_D^{20}$ − 18.3 (*c* 0.30, MeOH); HR-ESI-MS and ^{1}H and ^{13}C NMR spectral data were consistent with those of hydroxyeucomic acid in the literature [7].

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